In vitro and epidemiological studies of sweet potato (Ipomoea batatas) (L.) Lam. virus diseases in Kenya

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IN VITRO AND EPIDEMIOLOGICAL STUDIES OF SWEET POTATO (*IPOMOEA BATATAS*) (L.) LAM. VIRUS DISEASES IN KENYA

Submitted by

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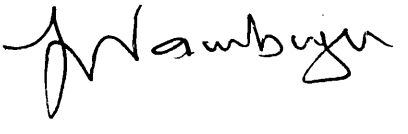

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Dedication

To the memories of my late mother Elizabeth W. Kanduthu,
my sister Rosalid W. Mathenge, my husband Francis
K. Wambugu, my children Benson Wambugu, James Kanduthu,
and Marybeth Nyokabi, for their love and support over years.

Abstract

In surveys of sweet potato crops in Kenya, viruses were found to be prevalent and to occur in complexes in all production areas. Kenyan isolates of sweet potato feathery mottle virus (SPFMV), sweet potato mild mottle virus (SPMMV) and sweet potato caulimo-like virus (SPC-LV) were isolated and partially characterized; other viruses (sweet potato latent virus, sweet potato chlorotic stunt virus, sweet potato ringspot virus and cucumber mosaic virus) were detected by serological procedures but have yet to be investigated further. Severe symptoms in sweet potato were associated with SPMMV and possibly a "virus-like agent" (VLA) similar to those reported in West Africa (Nigeria) and Israel.

Re-infection studies with for example SPFMV showed that, the most rapid rate of re-infection in the first crop season (25%) occurred in Western Kenya, less rapid (14%) in Eastern Kenya and none (0%) elsewhere. In the second season about 50% of re-infection by all viruses had occurred in Eastern and Central Kenya, and 70% in Western Kenya; by the end of third season upto 95% re-infection was recorded. The extent of re-infection was associated with seasonal occurrence of the aphid or whitefly vectors of the viruses.

In Central Kenya, where the sweet potato crop is grown on perennial basis, an isolation distance of c. 50 m was effective in controlling re-infection (to less than 10%) during the first year, but subsequently was not effective. Prevailing wind direction had some effect (a re-infection difference of about 12%) but it was not strong.

Two improved procedures were developed for virus elimination from sweet potato vegetative germplasm: *in vitro* thermotherapy (40-30 °C in the light-dark daily cycles respectively, of a 16 hr photoperiod, for 4 weeks) using MS basal medium

followed by meristem-tip culture (MTC) and *in vitro* chemotherapy using 2,4-Dioxohexahydro-1, 3, 5-Triazine (DHT), Ribavirin (RB) (60 and 40 mg/l respectively, for 4 weeks) with MTC.

Regeneration studies showed that plants could be regenerated from primary callus derived from *in vitro* lateral root explants using MS basal medium with Kn 0.2 or 0.4 mg/l. Plants were also regenerated from *in vivo* or *in vitro* leaf-derived callus cultures (callus induced with 1.0 mg/l 2,4-D), using MS basal medium with chemotherapeutants DHT 100 mg/l + RB 10 mg/l or DHT 100 mg/l alone, for 4 weeks, followed by transfer to medium with Zn 0.25 mg/l + IAA o.5 mg/l for 4 weeks, and a final transfer to high sucrose (60 mg/l) hormone-free MS basal medium. Preliminary studies with isolated leaf cells showed that callus colonies could be produced on MS basal medium containing Zn 0.25 mg/l and IAA 0.5 mg/l but plant regeneration was not achieved.

Contents

	Page
Acknowledgements	
Dedication	
Abstract	i
Contents	iii
List of Tables	x
List of Figures	xiii
Abbreviations	xxi
CHAPTER 1 - GENERAL INTRODUCTION	
1.1- Importance of Sweet Potato World-wide	1
1.2- Importance of Sweet Potato in Eastern and Central Africa	8
1.3- Importance and Distribution of Sweet Potato Crops in Kenya	12
1.4- The Origin and Botany of *Ipomoea batatas*	16
1.5- Major Constraints to Production of Maximum Yields	17
1.6- Research Objectives	20
CHAPTER 2 - GENERAL MATERIALS AND METHODS	
2.1- Plant Materials	21
2.2- Plant Growth and Maintenance in the Greenhouse	21
2.3- General Culture Media Preparation	22
2.4- General Culture Techniques and Incubation	25
2.5- Virus Detection and Identification	25
2.5.1- Transmission to Experimental Hosts	26
2.5.1.1- Graft-inoculation	26

2.5.1.2- Mechanical-inoculation 26
2.5.2- Electron Microscopy 27
2.5.3- Immunosorbent Electron Microscopy (ISEM) 27
2.5.4- Enzyme-linked Immunosorbent Assay, Double Antibody Sandwich (DAS-ELISA) 28
2.5.5- Enzyme-Linked Immunosorbent Assay on Nitrocellulose Membrane (NCM-ELISA) 30
2.5.6- Double Gel-diffusion Test 32
2.5.7- Virus Purification 32
CHAPTER 3 - STUDIES OF SWEET POTATO VIRUSES
3.1- General Introduction 33
3.1.1- Viruses known to infect sweet potato 34
3.1.1.1- Viruses with Filamentous Particles 34
3.1.1.1.1- Sweet Potato Feathery Mottle Virus (SPFMV) 34
3.1.1.1.2- Sweet Potato Latent Virus (SPLV) 35
3.1.1..1.3- Sweet Potato Mild Mottle Virus (SPMMV) 36
3.1.1.1.4- Sweet Potato Chlorotic Stunt Virus (SPCSV) 37
3.1.1.1.5- Sweet Potato Yellow Dwarf Virus (SPYDV) 37
3.1.1.1.6- Sweet Potato Vein Mosaic Virus (SPVMV) 38
3.1.1.2- Viruses With Isometric Particles 38
3.1.1.2.1- Sweet Potato Caulimo-Like Virus (SPC-LV) 38
3.1.1.2.2- Sweet Potato Ringspot Virus (SPRSV) 39
3.1.1.2.3- Cucumber Mosaic Virus (CMV) 39
3.1.1.2.4- Sweet Potato Leaf Curl Virus (SPLCV) 40
3.1.1.3- Sweet Potato Virus-Like Agent (SP-VLA) 40
3.1.1.4- Other Virus Isolated From Sweet Potato 41

3.1.1.5- Uncharacterised Sweet Potato Viruses 41
3.2- Materials and Methods 42
3.2.1- Re-infection of Clean Sweet Potatoes Germplasm 42
3.2.1.1- Experimental sites 42
3.2.1.2- Planting materials 42
3.2.1.3- Experimental design 43
3.2.1.4- Virus Identification 45
3.2.2- Effects of Distance and Prevailing Southerly Wind on Spread of Sweet Potato Viruses 46
3.2.2.1- Experimental Site, materials and design 46
3.2.2.2- Virus Assay 48
3.2.3- Monitoring of Aphids and Whiteflies 48
3.3- Results of Virus Studies 50
3.3.1- Surveys, identification and Characterization of Kenyan Viruses 50
3.3.1.1- Results of Visual Surveys for Sweet Potato Virus Disease symptoms in Kenya and Central Uganda 50
3.3.1.2- Viruses detected in Sweet Potato Samples Collected During Surveys 60
3.3.1.2- Partial Characterisation of some Kenyan Viruses (SPFMV, SPMMV, SPC-LV, SP-VLA) 72
3.3.2- Epidemiology 97
3.3.2.1- Re-infection 97
3.3.2.2- Spread of Sweet Potato Viruses in Relation to Distance from Foci of Infection at Muguga 104

3.3.2.3- Spread of Sweet Potato Viruses in Relation to Prevailing Wind Direction at Muguga 110
3.3.3- Aphid and Whitefly Trapping 115
3.3.3.1- Trapping of Aphid Species and Relationship with Rainfall and Cropping Season 115
3.3.3.2- Catches of Known Virus Vector Aphid Species 124
3.3.3.3- Whitefly Catches by Use of 3 Metre Sticky Yellow Column Traps 127
3.3.3.4- Whitefly Catches in Yellow Water Traps (January-November 1990) 132
3.4- Discussions 134
CHAPTER 4 - CHEMOTHERAPY AS AN ADJUNCT TO MERISTEM-TIP CULTURE FOR OBTAINING VIRUS-FREE SWEET POTATOES
4.1- Introduction 139
4.2- Materials and methods 143
4.2.1- Sweet Potato Genotypes, Maintenance and Culture Methods 143
4.2.2- Chemotherapeutants 143
4.2.3- Meristem-tip Culture (MTC) 144
4.2.4- Shoot-tip culture (STC) 144
4.2.5- Media Preparation and Culture Incubation 144
4.2.6- Virus Testing 145
4.2.7- Field Experiments (in Kenya) 145
4.2.8- Statistical Analysis of Data 147
4.3- Results 148

4.3.1- The Effects of Different Chemotherapeutants on Meristem-tip (MTC) and Shoot-tip Cultures (STC) 148
4.3.2- Effects of 2-Thiouracil on MTC 148
4.3.3- Effects of Vidarabine on MTC 149
4.3.4- Effects of Combined Chemotherapeutants on MTC 154
4.3.5- Effects of Ribavirin on MTC and Field-grown MTC-derived Plants 158
4.3.6- Effects of DHT on MTC 162
4.3.7- Effects of DHT on Shoot-tip Culture 166
4.4- Discussions 172
4.4.1- Aims of These Studies 172
4.4.2- 2-Thiouracil 172
4.4.3- Vidarabine 173
4.4.4- DHT and Ribavirin 173
4.4.5- Conclusions 175
CHAPTER 5 - TISSUE CULTURE STUDIES
5.1- Introduction 177
5.2- Materials and Methods 177
5.2.1- *In vitro* Thermotherapy 177
5.2.2- Meristem-tip Culture (MTC) 178
5.2.3- Acclimatization to Soil Conditions 179
5.2.4- Virus Indexing 181
5.3- Results 181
5.4- Discussions 183

CHAPTER 6 - REGENERATION OF SWEET POTATO FROM CELL CULTURES

6.1- Introduction 186

6.2- Materials and Methods 189

6.2.1- Root Cultures 189

6.2.2.1- Leaf Culture 189

6.2.2.1- Callus Induction from Leaf-explant 189

6.2.2.2- Callus induction from *in vitro* shoots 190

6.2.2.3- Regeneration Procedures 190

6.2.2.4- Testing for Virus Elimination 191

6.3- Results 192

6.3.1- Root Culture Regeneration 192

6.3.2- Leaf Culture Regeneration 197

6.3.2.1- Callus Induction 197

6.3.2.2- Shoot Regeneration By use of Hormones BA/NAA system 198

6.3.2.3- Effects of Hormones and Chemotherapeutants on Regeneration and Virus Elimination 205

6.3.2.3.1- Effects of Chemotherapeutants on callus formation 205

6.3.2.3.2- Effects of Combinations of Hormones and Chemotherapeutants in Single Stage Application on Callus Production, Regeneration and Virus Elimination 207

6.3.2.3.3- Effects of Chemotherapeutants and Hormones Following Two Further Stages 208

6.3.2.3.4- Results from Tests for Virus Elimination: 215

6.3.3- Callus Induction from Isolated Leaf Cells 216
6.3.3.1- Introduction 216
6.3.3.2- Isolation of Cells from Leaves 217
6.3.3.3- Cell Plating 218
6.3.3.4- Results 219
6.4- Discussions 226
6.4.2- Plant Regeneration from Root Callus 228
6.4.3.1- Plant Regeneration Following the Use of NAA, BAP and GA_3 229
6.4.3.2- Plant Regeneration Following the Use of Zn and 1AA, and Chemotherapeutants (DHT and RB) 230
6.4.4- Mesophyll Cell Isolation and Callus Development: 232
CHAPTER 7 - GENERAL CONCLUSION
7.1 Overall Aim of the Research 234
7.1.1 Production of Virus-free Stocks 234
7.1.2 Cell Culture Studies 238
7.1.3 Control Strategies for Spread of Virus Diseases in Field 239
7.1.4 Conclusions 240
Appendices
Appendix I 243
Appendix II 244
References 246

List of Tables

Table 1 - Production and economic values of 10 important food crops in developing countries (1980-82)

Table 2 - World sweet potato production 1983-85 and changes since 1961-63.

Table 3 - Nutritional value of sweet potato tubers and leaves

Table 4 - Comparative calorific values of cereals and non-cereal crops

Table 5 - Sweet potato production in East Africa

Table 6 - Composition of the Murashige and Skoog (1962) medium

Table 7 - Composition of International Potato Centre (CIP) media for sweet potato in vitro cultures

Table 8 - Genotypes used in re-infection experiments

Table 9 - Visual surveys for sweet potato virus diseases in Kenya and Uganda

Table 10 - Relative occurrence of seven viruses in areas surveyed in Kenya and Uganda

Table 11 - Relative occurrence of seven viruses of sweet potatoes detected in field surveys in Kenya and Uganda

Table 12 - Samples (%) with multiple virus infections in areas surveyed

Table 13 - Infection of sweet potatoes with virus complexes in Kenya and Uganda

Table 14 - Frequency of CMV in relation to that of SPFMV and SPLV

Table 15 - Frequency of CMV in relation to that of SPFMV and SPLV summarised by province

Table 16 - SPC-LV Immunosorbent electron microscopy

Table 17 - Levels (%) of re-infection at four experimental locations, August 1989 to November 1990.

Table 18 - Spread of sweet potatoes viruses in relation to distance from foci of infection at Muguga

Table 19 - Spread of sweet potatoes viruses at Muguga in relation to prevailing southerly wind direction

Table 20 - Aphid species caught in yellow water traps in four different locations in Kenya (Dec. 1989 - Nov. 1990)

Table 21 - Mean monthly rainfall (mm) for Embu, Kakamega, Katumani and Muguga during the insect monitoring period (Dec. 1989 - Nov. 1990)

Table 22 - Whiteflies caught (no.) on sticky yellow column traps (Dec. 1989 - Nov. 1990)

Table 23 - Whiteflies caught (no.) in yellow water traps (Jan. - Nov. 1990)

Table 24 - Stages of plantlet regeneration from meristem-tip (MTC) or shoot-tip cultures (STC)

Table 25 - Effects of Vidarabine concentrations on virus elimination and growth in vitro of sweet potato plantlets derived from MTC

Table 26 - Effects of combined chemotherapeutants on virus elimination and growth of cv. Papota and genotype HIB 687

Table 27 - Effects of ribavirin on growth (in vitro and in field) and virus elimination of cv. Papota and genotype HIB 687

Table 28 - Effects of DHT on growth (in vitro and in field) and virus elimination of cv. Papota and genotype HIB 687

Table 29 - Effects of DHT on growth and virus elimination of shoot-tip cultures (STC)

Table 30 - Summary of procedure for in vitro thermotherapy and meristem-tip culture (MTC)

Table 31 - In vitro thermotherapy followed by meristem-tip culture (MTC) technique and virus assay

Table 32 - Effects of different combinations of hormone, RB and DHT on morphogenetic responses of roof cultures

Table 33 - Effects of 2,4-D on callus induction from leaf sections from different sweet potato genotypes

Table 34 - Effects of different hormone combinations on the morphogenetic responses of sweet potato callus

Table 35 - Summary of responses observed during shoot regeneration from leaf-derived callus using chemotherapeutants, hormones and high sucrose concentration

Table 36 - Composition of cell isolating and plating media

Table 37 - Effects of Zeatin and IAA on growth and development of sweet potato leaf cell-derived callus

List of Figures

Fig. 1. World production of major crops.

Fig. 2. Sweet potato production; area and yield in developing countries 1961-87 (3 - year moving average).

Fig. 3. Sweet potato production trends in developing countries of Africa, Asia, Latin America and in developed countries (3 - year moving averages).

Fig. 4. Sweet potato production (annual means 1985-1988) in Eastern and Southern African countries.

Fig. 5. Sweet potato production areas in Kenya.

Fig. 6. Production (mean area) of sweet potato in Kenyan Provinces 1985-1986.

Fig. 7. Increase (%) in production 1961/65 (average) to 1981 of 10 important food crops in relation to population increase in developing countries.

Fig. 8. Experimental layout of the field trial at Mguga to study the effects of distance and prevailing wind on virus spread.

Fig. 9. Areas of Kenya surveyed for sweet potato virus diseases, 1989-1990.

Fig. 10. Surveys showing the common occurrence in sweet potatoes of virus complexes in Coast Province.

Fig. 11. Surveys showing the common occurrence in sweet potatoes of virus complexes in Eastern Province.

Fig. 12. Surveys showing the common occurrence in sweet potatoes of virus complexes in Central Province.

Fig. 13. Surveys showing the common occurrence in sweet potatoes of virus complexes in Rift-Valley Province.

Fig. 14. Surveys showing the common occurrence in sweet potatoes of virus complexes in Western and Nyanza Provinces.

Fig. 15. Surveys showing the common occurrence in sweet potatoes of virus complexes in Central Uganda.

Fig. 16I. Symptoms induced by sweet potato caulimo-like virus (SPC-LV) in *Ipomoea setosa* after graft transmission from sweet potato.

a. Initial symptoms of general chlorosis after graft transmission.

b. Broad vein chlorosis.

c. Extensive leaf chlorosis leading to the death of the leaf.

16II. Particle morphology of SPC-LV.

a. SPC-LV. Purified virus "Bar scale represents 100 nm".

b. SPC-LV. Decorated particles "Bar scale represents 100 nm".

Fig. 17I. Symptoms induced by sweet potato mild mottle virus (SPMMV) in different sweet potato cultivars, of sweet potato, *Ipomoea setosa* and *Nicotiana spp.*

a. Symptoms in sweet potato.

b. Symptoms in sweet potato.

c. Symptoms in *Ipomoea setosa.*

d. Symptoms in *Nicotiana tabacum.*

e. Symptoms in *Nicotiana clevelandii.*

f. Symptoms in of severe stunting and distortion in *N. clevelandii.*

17II. Particle morphology of SPMMV.

a. SPMMV. Partially purified preparation "Bar scale represents 300 nm".

Fig. 18. Symptoms induced by sweet potato featherly mottle virus (SPFMV) in different cultivars of sweet potato.

a. maroon spots and veins.

b. chlorotic spots with maroon margins.

c. maroon vein-banding.

d. few chlorotic spots on leaf (cv. Rose Centennial).

e. numerous chlorotic spots.

f. chlorotic spots developing maroon margins.

Fig. 19I. Symptoms induced by SPFMV in *Ipomoea setosa* after graft transmission.

a. Initial symptoms of chlorotic spotting followed by vein banding after graft transmission to *I. setosa.*

b. Symptoms of chlorotic vein banding on mature leaf of *I. setosa.*

c. Symptoms of SPFMV infected *Ipomoea nil* after mechanical transmission.

19II. Particle morphology of SPFMV.

a. SPFMV. Particles in leaf extract of infected Ipomoea setosa mounted in 2% neutral potassium phosphotungstate. "Bar scale represents 250 nm".

Fig. 20. Symptoms induced by sweet potato mild mottle virus and virus-like agent complex (SPMMV+VLA) in sweet potato and *Ipomoea setosa.*

a. Symptoms in sweet potato.

b. *Ipomoea setosa* showing severe symptoms of shoe-stringing.

c. Symptoms in *Ipomoea setosa* showing chlorosis and severe distortion.

d. Healthy (left) infected (right) *Ipomoea setosa* showing shoe-stringing.

e. Detailed healthy (left) and infected (right) *Ipomoea Setosa.*

Fig. 21. Levels (%) of re-infection at Embu by SPFMV, SPMMV, SPLV, CMV and SPCSV.

Fig. 22. Levels (%) of re-infection at Kakamega by SPFMV, SPMMV, SPLV, CMV and SPCSV.

Fig. 23. Levels (%) of re-infection at Katumani by SPFMV, SPMMV, SPLV, CMV and SPCSV.

Fig. 24. Levels (%) of re-infection at Muguga by SPFMV, SPMMV, SPLV, CMV and SPCSV.

Fig. 25. Spread of SPFMV in relation to distance from foci of infection at Muguga.

Fig. 26. Spread of SPMMV in relation to distance from foci of infection at Muguga.

Fig. 27. Spread of SPLV in relation to distance from foci of infection at Muguga.

Fig. 28. Spread of SPC-LV in relation to distance from foci of infection at Muguga.

Fig. 29. Spread of CMV in relation to distance from foci of infection at Muguga.

Fig. 30. Spread of SPCSV in relation to distance from foci of infection at Muguga.

Fig. 31. Prevailing southerly wind direction and spread of SPFMV at Muguga.

Fig. 32. Prevailing southerly wind direction and spread of SPMMV at Muguga.

Fig. 33. Prevailing southerly wind direction and spread of SPLV at Muguga.

Fig. 34. Prevailing southerly wind direction and spread of SPC-LV at Muguga.

Fig. 35. Prevailing southerly wind direction and spread of CMV at Muguga.

Fig. 36. Prevailing southerly wind direction and spread of SPCSV at Muguga.

Fig. 37. Catches of known vector and other aphid species at Embu (Dec. 1989 - Nov. 1990).

Fig. 38. Mean monthly rainfall (mm) for Embu during the insect monitoring period (Dec. 1989 - Nov. 1990).

Fig. 39. Catches of known vector and other aphid species at Kakamega (Dec. 1989 - Nov. 1990).

Fig. 40. Mean monthly rainfall (mm) for Kakamega during the insect monitoring period (Dec. 1989 - Nov. 1990).

Fig. 41. Catches of known vector and other aphid species at Katumani (Dec. 1989 - Nov. 1990).

Fig. 42. Mean monthly rainfall (mm) for Katumani during the insect monitoring period (Dec. 1989 - Nov. 1990).

Fig. 43. Catches of known vector and other aphid species at Muguga (Dec. 1989 - Nov. 1990).

Fig. 44. Mean monthly rainfall (mm) for Muguga during the insect monitoring period (Dec. 1989 - Nov. 1990).

Fig. 45. Catches of four known aphid vector species of SPFMV at Embu.

Fig. 46. Catches of four known aphid vector species of SPFMV at Kakamega.

Fig. 47. Catches of four known aphid vector species of SPFMV at Katumani.

Fig. 48. Catches of four known aphid vector species of SPFMV at Muguga.

Fig. 49. Whitefly catches at Embu (Dec. 1989 - Nov. 1990).

Fig. 50. Whitefly catches at Kakamega (Dec. 1989 - Nov. 1990).

Fig. 51. Whitefly catches at Katumani (Dec. 1989 - Nov. 1990).

Fig. 52. Whitefly catches at Muguga (Dec. 1989 - Nov. 1990).

Fig. 53. Effects of vidarabine on plant growth; sweet potato genotype 560 (HIB 687).

Fig. 54. Effects of vidarabine on plant growth (meristem-tip culture) of genotypes 157 HIB 687.

a. Effects of VID on the no. of internodes.

b. Effects of VID on the stem length (mm).

c. Effects of VID on the fresh weight (gm).

Fig. 55. Effects of different combinations of chemotherapeutants on plant growth of (meristem-tip culture) of cvs. Papota and HIB 687.

a. Effects on the no. of internodes.

b. Effects on the stem length (mm).

c. Effects on the fresh weight (gm).

Fig. 56. Effects of ribavirin on plant growth (meristem-tip culture) of cvs. Papota and HIB 687.

a. Effects of RB on the no. of internodes.

b. Effects of RB on stem length (mm).

c. Effects of RB on fresh weight (gm).

Fig. 57. Effects of DHT on plant growth (meristem-tip culture) of cvs. Papota and HIB 687.

a. Effects of DHT on the no. of internodes.

b. Effects of DHT on stem length (mm).

c. Effects of DHT on fresh weight (gm).

Fig. 58. Effects of DHT on plant growth (shoot-tip culture) of cvs. Papota and 560 (HIB 687).

a. Effects of DHT on cv. HIB 687 14 week old shoot-tip cultures.

b. Effects of DHT on cv. HIB 687 18 week old shoot-tip cultures.

c. Effects of DHT on cv. Papota 14 weeks old shoot-tip cultures.

Fig. 59. Effects of DHT on plant growth (shoot-tip culture) of cvs. Papota, HIB 687, TIB 9, and Rose Centennial.

a. Effects of DHT on the no. of internodes.

b. Effects of DHT on the stem length (mm).

c. Effects of DHT on the fresh weight (gm).

Fig. 60. Root culture on MS medium supplemented with 3% (w/v) sucrose and 0.2 mg/l Kn, incubated in the growth room (25 °C, 16 hr photoperiod 30 $\mu Mm^{-2}s^{-1}$PAR).

a. Explant at zero day (Mag x 7).

b. Plantlet after culture for eight weeks (Mag x 1).

Fig. 61. Shoot regeneration from leaf-derived callus using BA/NAA hormone system of cv. HIB 687.

a. Callus formed after 3 weeks of culture on MS medium supplemented with 3% (w/v) sucrose and 1.0 mg/l 2,4-D, incubated in the growth cabinet (27 °C in the dark) (Mag x 2).

b. Nodule formation 2 weeks after transfer of callus to MS medium

supplemented with 3% (w/v) sucrose, NAA 1.0, BA 0.1 and GA_3 10.0 mg/l, and incubated in the growth room (25 °C, 16 hr photoperiod 30 $\mu Mm^{-2}s^{-1}$PAR) (Mag x 4).

c. Further development of the nodules after 3 weeks of culture on the same medium (Mag x 3).

d. verticle root-like structures (adventitious roots) development from nodules after 4 weeks of culture on the same medium, ending with tiny pinkish swellings (Mag x 1).

e. Shoot-buds development at the base of adventitious roots 6 weeks after transfer to MS-medium (hormone-free) supplemented with 6% (w/v) sucrose (Mag x 3).

Fig. 62. Plantlet regeneration from leaf-derived callus using chemotherapeutants (RB + DHT) and Zn/IAA hormone system, cv. HIB 687.

a. Callus induction, same as Fig. 61a (Mag x 2).

b. Nodule formation 4 weeks after transfer of callus to MS medium supplemented with 3% (w/v) sucrose, RB 10 mg/l and DHT 100 mg/l, and incubated in the growth room (Mag x 4).

c. Further development of nodules and dormant shoot-buds 4 weeks after transfer to MS medium supplement with 3% (w/v) sucrose, Zn 0.25 mg/l and IAA 0.5 mg/l (Mag x 4).

d. Shoot-buds development 4 weeks after transfer of callus with nodules to MS medium (hormone-free) supplemented with 6% (w/v) sucrose (Mag x 3).

e. Further development of shoot-buds 6 weeks after transfer to the MS-medium (hormone-free) with 6% (w/v) sucrose (Mag x 2).

f. plantlets development 8 weeks after transfer of shoot-buds to half strength MS-medium (hormone-free) with 3% (w/v) sucrose (Mag x 2).

Fig. 63. Callus formation from isolated leaf cells.

63I. Cell colony formation from isolated leaf cells, cultured on plating medium (Table 36) and incubated first for one week in a growth cabinet (27 °C in the dark) then for three weeks in a growth room (25 °C, 16 hr photoperiod 30 $\mu Mm^{-2}s^{-1}$PAR).

a. inoculum (cells) at day zero (Mag x 372).

b. Small colonies 10 days after plating (Mag x 380).

63II. Colonies formed from isolated leaf cells, after calluses were transferred from plating medium to regeneration medium (M12, Table 37), and incubated in growth room.

a. 5 mm colony at day zero (Mag x 15).

b. Colonies development in a plate after 4 weeks of incubation (Mag x 1).

c. Details of one callus colony from b (Mag x 3).

Abbreviations

°C	degrees centigrade
2,4-D	2,4-Dichlorophenoxyacetic acid
2-Thio	2-thiouracil (4-Hydroxy-2-thiopyrimidine)
5 FOA	5 - Fluororotic acid
ABA	Abscisic acid
ACLSV	Apple chlorotic leaf spot virus
Ad	Adenine
AlfMV	Alfalfa mosaic virus
AMV	Arabis mosaic virus
AVRDC	Asian Vegetable Research and Development Centre
BA or BAP	6-Benzyladenine or 6-benzylaminopurine
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BSA	bovine serum albumin
c.	approximately
CATIE	Centro Agron Trop de Investigacion Ensenaza
CERV	Carnation etched ring virus
CIBC	Commonwealth Institute of Biological Control
CIMV	Cauliflower mosaic virus
CIP	International Potato Centre
CLRV	Cherry leaf roll virus
cm	centimetre
CMV	Cucumber mosaic virus
CV(s)	Cultivar(s)

DAS-ELISA	Double antibody sandwich form of enzyme-linked immunosorbent assay
DHT	2,4-D - dioxohexahydra - 1,3,5 -triazine
DIECA	Diethyldithio carbonate
DMSO	dimethyl sulphoxide
DMV	Dahlia mosaic virus
EA	East Africa
EDTA	Ethylenediaminetetra acetate
ELISA	Enzyme-linked immunororbent assay
EtOH	Ethyl alcohol
FAO	Food and Agricultural Organization
g	gram
GA_3	Gibberellic acid
hr	hour
HPS	High pressure sodium
HRI	Horticulture Research International
HRP	Horse radish peroxidase
IAA	Indole-3-acetic acid
ICIPE	International Centre of Insect Physiology and Ecology
ICRISAT	International Crop Research Institute for the Semi-arid Tropics
IgG	Immunoglobulins
IITA	International Institute of Tropical Agriculture, (Ibadan, Nigeria)
ISAB	Institut de Sciences Agronomiques de Burundi (at Bujumbura)
ISEM	Immunosorbent electron microscopy
KARI	Kenya Agricultural Research Institute (Kenya)
kg	kilogram

Km	kilometre
Kn	Kinetin (6 - furfurylaminopurine)
KS	Kenyan sweet potato cultivars
M_r	relative molecular mass
Mag	Magnification
MASL	meter above sea level
mg	milligram
min	minute
mm	millimetre
MTC	Meristem-tip culture
NAA	Naphthalene-acetic acid (1-Naphthaleaneacetic acid)
NaOH	Sodium hydroxide
NARC	National Agricultural Research Centre
NBT	nitroblue tetrazolium
NCM	Nitrocellulose membrane
NCM-ELISA	Enzyme-linked immunosorbent assay on nitrocellulose membrane
nm	nanometer
NPK	Nitrogen phosphorus potassium fertilizer
PAR	Photosynthetically active radiation
PDV	Prune dwarf virus
PL-RV	Potato leafroll virus
PNRV	Prunus necrotic ringspot virus
PQS	Plant Quarantine - KARI, Kenya
PVM	Potato M virus
PVP	Polyvinylpyrrolidone

PVS	Potato S virus
PVX	Potato X virus
PVY	Potato Y virus
RB	(Ribavirin=Virazole)-1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide
RC	Rose centennial
RNA	Ribonucleic acid
RRC	Regional Research Centre
SoyCMV	Soybean chlorotic mottle virus
SP-VLA	Sweet potato virus-like agents
SPC-LV	Sweet potato caulimo-like virus
SPCSV	Sweet potato chlorotic stunt virus
SPFMV	Sweet potato feathery mottle virus
SPLCV	Sweet potato leaf curl virus
SPLV	Sweet potato latent virus
SPMMV	Sweet potato mild mottle virus
SPRSV	Sweet potato ringspot virus
SPVMV	Sweet potato vein mosaic virus
SPYDV	Sweet potato yellow dwarf virus
STC	Shoot-tip culture
StCV	Strawberry crinkle virus
StMV	Strawberry mottle virus
TBS	Tris - buffered saline (20 mM tris, 500 mM Nacl, 20 mM NaN_3, pH 7.5)
TMV	Tobacco mosaic virus
TTBS	Tris-buffered saline containing 0.05% (v/v) Tween 20
VID	Vira-A = Vidarabine (Adenine 9-B-D-Arabinofuranoside)

yr	year
Zn	Zeatin (6-[4-Hydroxy-3-methylbut-2-enylamino] purine)

CHAPTER -1-

GENERAL INTRODUCTION

1.1 Importance of Sweet Potato World-wide

Tropical tuber crops such as sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), yams (*Dioscorea* spp.) and edible aroids (*Colocasia esculenta and Xanthosoma* spp.) are important food sources world-wide, but especially so in some developing tropical countries in which humid climates preclude the production of adequate yields of cereal crops. The total production of these crops in 1975 was estimated to exceed 140 million tonnes which provided food for more than 400 million people (Martin, 1984).

The global importance of sweet potatoes (Fig. 1) is now exceeded only by cereals (wheat, rice, maize and barley) and potatoes (Vietmeyer, 1986) and, of root and tuber crops, it is second in importance only to Irish or white potato *(Solanum tuberosum*) (Horton, 1988). However, in developing countries sweet potato is more important than potato (Table 1); although the crop is grown in over 100 countries, c. 80% is grown in Asia, just under 15% in Africa and almost 6% in the rest of the world (Table 2) (Horton, Prain and Gregory, 1989).

China is the world's largest producer of sweet potatoes (Fig. 2); it produces average yields which, in 1983-85, were threefold greater than those of developing countries in Africa, North and Central America and Oceania, and the two and half times more than the average production of all other countries (Table 2, & Fig. 2). Nevertheless, production in Africa is now almost 80% greater than in 1961-63 (Table 2, & Fig. 3).

Although sweet potatoes can be used in dried or fresh form as animal feed and

for the industrial production of starches, flour and alcohol, their major use is as human food; tubers are mostly eaten, but the leaves of some cultivars are consumed in Asia (Villareal *et al.*, 1979 a, b, & c). Both tubers and leaves are very nutritious (Table 3).

The nutritional value of sweet potatoes relative to the duration of production and calorific content is, even at present low levels of productivity in some areas, superior to that of other major cereals and non-cereal food crops (Table 4).

Table 1. PRODUCTION AND ECONOMIC VALUE OF 10 IMPORTANT FOOD CROPS IN DEVELOPING COUNTRIES (1980-82)

Crop	Production ($tx10^6$)*	Area ($hax10^6$)	Yield (t/ha)	Value ($x10^9$ US $)*
Rice	382.6	139.5	2.7	65.0
Wheat	162.4	142.1	1.1	24.0
Maize	153.9	78.3	2.0	18.3
Potato	91.1	8.7	10.5	12.9
Sweet Potato	137.0	9.3	14.7	12.2
Cassava	127.4	14.4	8.9	8.9
Banana +Plantain	61.9	no data	no data	6.6
Sorghum	43.7	40.1	1.1	5.4
Groundnut	16.9	18.0	0.9	5.0
Millet	20.9	40.2	0.7	3.9

Statistics from Horton (1988)

* Estimates of farm-level prices for 1977 from FAO.

Table 2. WORLD SWEET POTATO PRODUCTION (1983-1985)
AND CHANGES SINCE 1961-1963

	1983/1985 Average				change in production (%)
	Production (ton x 10^3)	Yield (ton ha^{-1})	Harvested area (ha x 10^3)	Production per capital (kg)	
World	114,185	14	7,998	24	+13
Asia	104,603	16	6,413	38	+12
(China)	93,550	18	5067	91	+23
Africa	6,100	6	1094	11	+78
North and Central America	1,442	7	213	4	+10
South America	1371	9	153	5	-37
Oceania	560	5	116	23	+52
Europe	108	11	10	0	-44
Developing countries Total	111,979	14	7867	32	+20
Developed countries Total	2,206	17	131	2	-70

From Horton, Prain and Gregory, 1989

Table 3. NUTRITIONAL VALUE OF SWEET POTATO TUBERS AND LEAVES

	Nutritional value* of	
	Tubers	Leaves
Calories	108.00-121.00	42.00
Moisture (%)	73.30-68.80	86.70
Protein (g)	1.00-1.90	3.20
Fat (g)	0.30-0.20	0.70
Carbohydrates (g)	25.60-28.50	8.00
Fibre (g)	0.80-1.00	1.60
Ash (g)	0.70-1.00	1.40
Calcium (mg)	21.00-33.00	86.00
Phosphorous (mg)	38.00-50.00	81.00
Iron (mg)	0.90-2.00	4.50
Sodium (mg)	31.00	5.00
Potassium (mg)	210.00	562.00
B-Carotene (mg)	35.00-2400.00	2215.00
Thiamine (mg)	0.09-0.14	0.11
Riboflavin (mg)	0.04-0.05	0.22
Niacin (mg)	0.70	0.70
Ascorbic acid (mg)	21.00-37.00	17.00

* Nutrients in 100 g of edible material.

Data from Martin (1984).

Table 4. COMPARATIVE CALORIFIC VALUES OF CEREALS AND NON-CEREAL CROPS

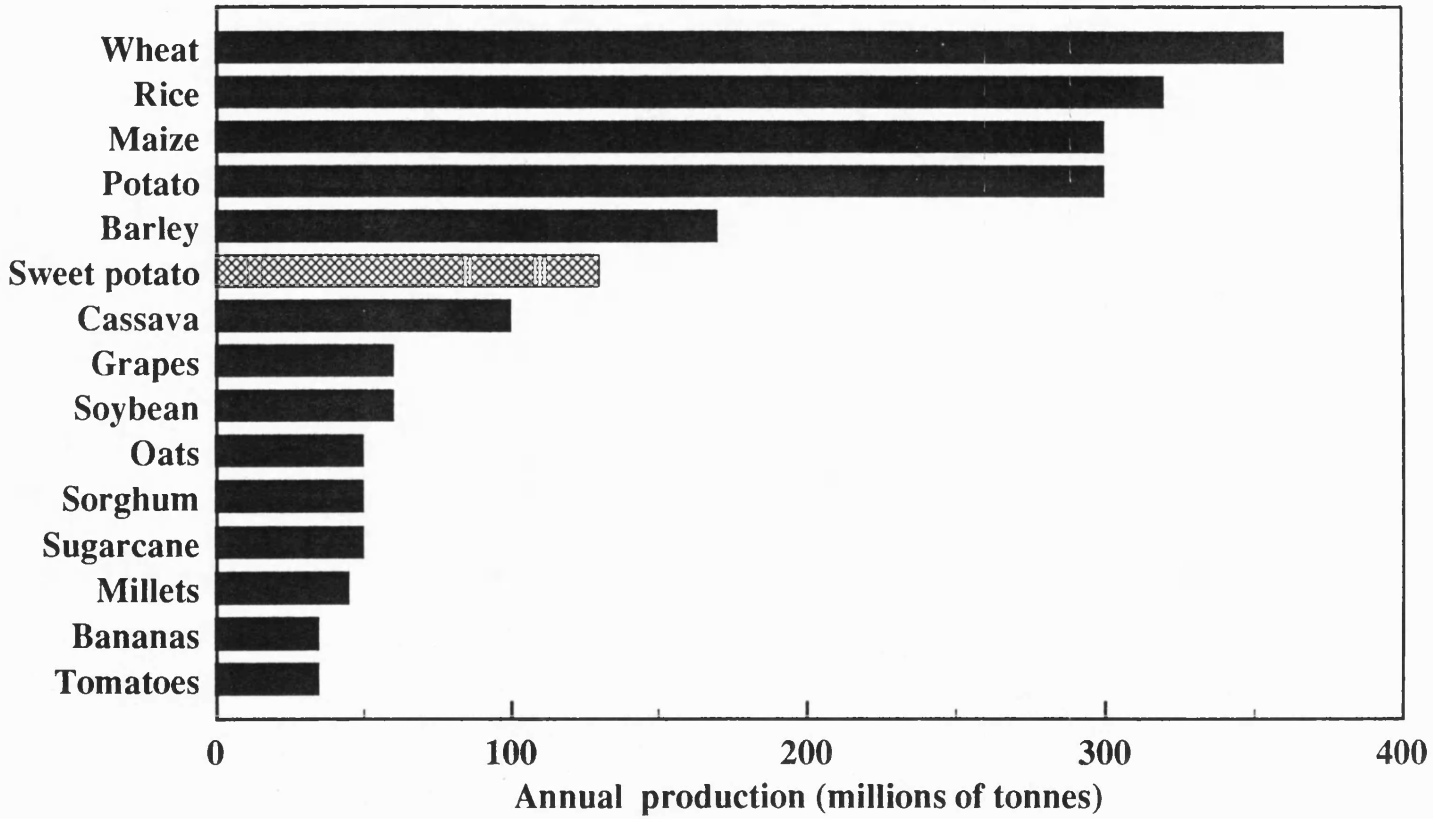
				EDIBLE ENERGY			
		Average yield t-ha $^{-1}$	Crop maturity (days)	MJ/Kg	Proportion (%)	Per ha (MJx10 ^{3})	ha $^{-1}$ day $^{-1}$ (MJ)
Cereals*	Rice	2.01**	140	14.8	70	20.8	149
	Maize	1.24	130	15.2	100	18.8	145
	Sorghum	0.83	110	14.9	90	11.1	101
	Millet	0.55	100	15.0	100	8.2	82
Non-Cereals* (**)	Cassava	8.72	330	6.3	83	45.6	138
	Sweet Potato	6.45	140	4.8	88	27.2	194
	Yam	7.00	280	4.4	85	26.2	94
	Banana	13.60	365	5.4	59	41.4	113

After de Vries *et al.* (1967)

* Cereals, air-dried; non-cereals, fresh.

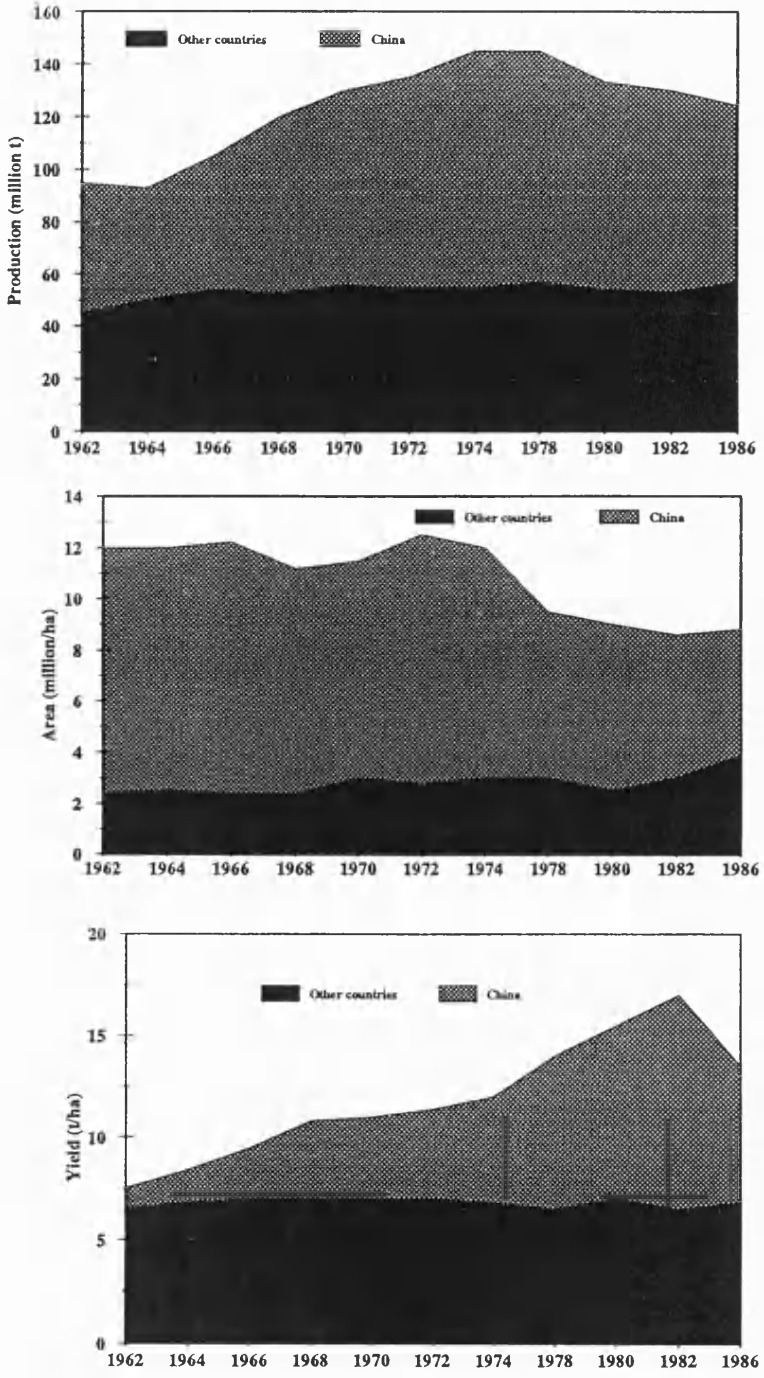
** Paddy.

Fig. 1. WORLD PRODUCTION OF 15 MAJOR CROPS



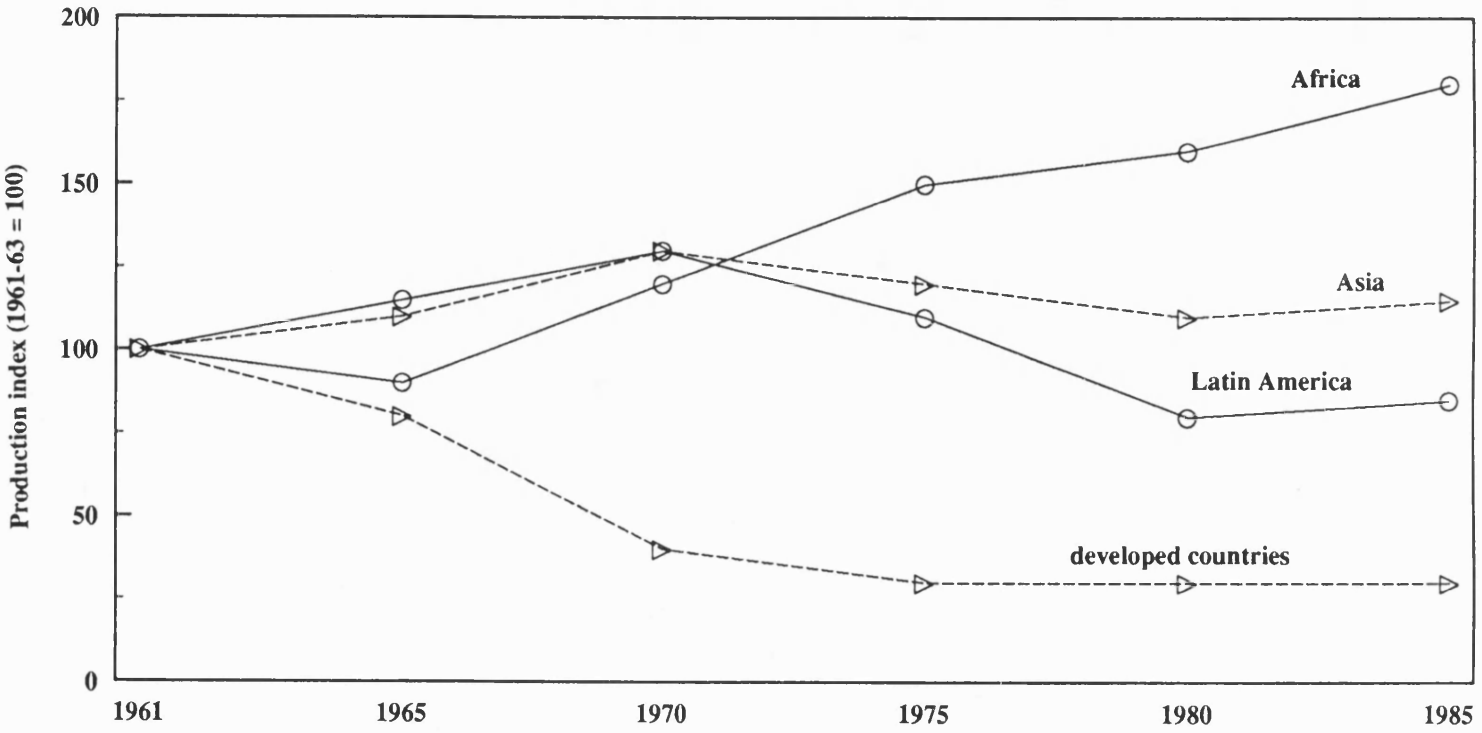
From Vietmeyer (1986)

Fig. 2. SWEET POTATO PRODUCTION; AREA AND YIELD IN DEVELOPING COUNTRIES 1961-1987
(3 year moving averages)



From: Horton, Prain & Gregory (1989)

Fig. 3. SWEET POTATO PRODUCTION TRENDS IN DEVELOPING COUNTRIES OF AFRICA, ASIA, LATIN AMERICA AND IN DEVELOPED COUNTRIES(3-YR MOVING AVERAGES)



Statistics from Horton (1988)

1.2 Importance of Sweet Potato in Eastern and Central Africa

Sweet potato is one of the more important food crops in East and Central Africa, being especially important in Uganda, Rwanda, Burundi, Madagascar, Zaire, Kenya and Tanzania (Fig. 4a and 4b). Statistics for land use, yield and total production in East Africa are presented in Table 5. The production of sweet potatoes gradually increased in Kenya and Uganda from 1969-79 (Table 5), but remained constant in Tanzania between 1977 and 1979. The increase in Kenya was relatively small when compared to the national food requirement, and production has not since increased. The major problems of sweet potato cultivation in Eastern Africa are attributable to the need for new improved adapted cultivars and the gradual degeneration of older cultivars probably due to virus infection (Hollings *et al.*, 1976; Sheffield, 1957; Mukiibi, 1977; Jana, 1982).

Planting virus-infected stem cuttings, the only material currently available, is probably the major reason for the widespread occurrence of infection and subsequent cultivar degeneration (see also 3.3.1.1 and 3.3.1.2).

Past research on sweet potato in East Africa was mainly by expatriate staff (Hollings *et al.*, 1976; Sheffield, 1957; Aldrich, 1963; Ingram, 1967; Macdonald, 1963; Wheatley, 1961). Such research involved surveys and identification of important diseases (including those caused by viruses), and the collection and selection of virus-tolerant and agronomically acceptable cultivars. Unfortunately, much of this research was not further developed or widely applied, and much of it has had very little impact on local farming practices. Although much needed, there is at present little research on sweet potato crops in East Africa. At an international level, the most recent report from Uganda was that by Mukiibi (1977) and from Tanzania by Jana (1982). At a national level, by contrast, the relevant government research institutions are very active; these activities include the national Root and Tuber Crops

Improvement Programmes at Ukirigura in Tanzania, the Kenya Agricultural Research Institutes (KARI) in Kenya, and the Namulonge Research Station in Uganda, the main aims of which are to provide improved local cultivars and to help farmers to increase overall production.

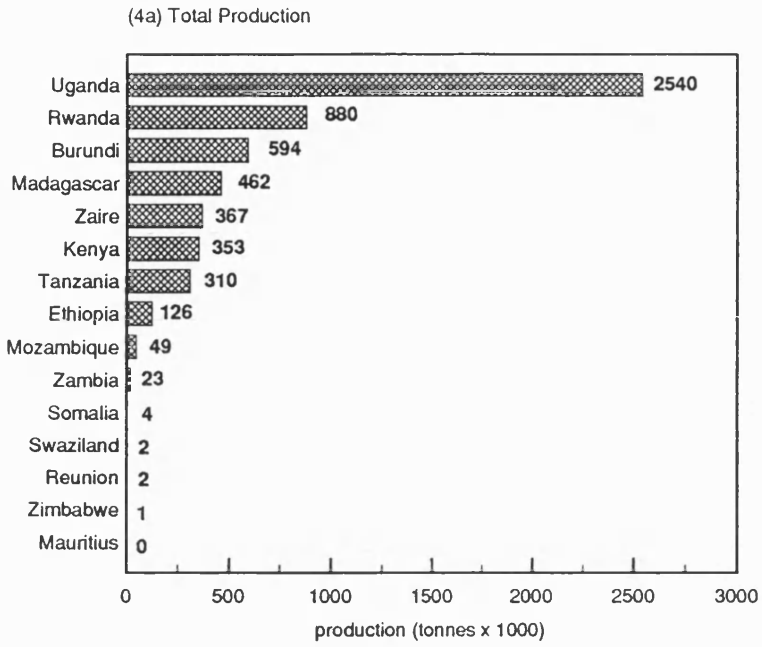
Table 5. SWEET POTATO PRODUCTION IN EAST AFRICA

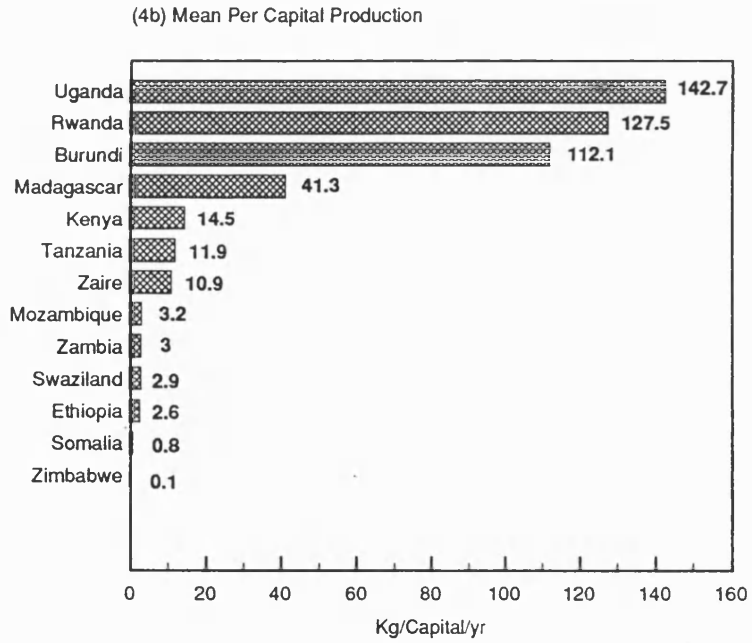
		Tanzania	Kenya	Uganda	All Africa
Area	1969-71	39	30	133	675
(hax10 ^{3})	1977	54*	36	137*	749
	1978	53*	37	139*	768
	1979	53	38*	140*	782
Yield	1969-71	6053	7753	5200	5954
(kg ha $^{-1}$)	1977	6204	7176	4818	6258
	1978	6226	8884	4865	6383
	1979	6226	8947	4865	6459
Total Production	1969-71	234	230	693	4020
(tx 10 ^{3})	1977	335*	258	660*	4688
	1978	330*	330	674*	4905
	1979	330*	340*	689*	5053

Source: Anon. (1980)

* FAO estimate.

Fig. 4. SWEET POTATO PRODUCTION (ANNUAL MEAN 1985-1988) IN EASTERN AND SOUTHERN AFRICAN COUNTRIES





Statistics for Ethiopia are Ministry of Agriculture estimates; others from FAO (Anon., 1989)

1.3 Importance and Distribution of Sweet Potato Crops in Kenya

The important tuber food crops in Kenya are potato, cassava and sweet potato but, of these three, sweet potato is the most widely distributed crop (Fig. 5). It is grown in most areas that do not exceed elevations of 2100 metres above sea level and receive sufficient rainfall for plants to survive (Jana, 1982). Of a production area of c. 34,000 ha in Kenya (Fig. 6), more than half (53.2%) is in Nyanza province and almost a quarter (24.7%) in the Western Province; other less important production areas are in the Central (10.6%), Eastern (8.8%) and Coast (2.7%) Provinces.

In the Central Province, sweet potatoes are usually grown for human food and animal feed in small holdings of less than 0.5 acres, the same plots often being used for 2-6 successive crops. When production of animal feed has the higher priority, cultivars are grown that produce copious amounts of foliage and stems but fewer tubers. The Central Province has climatic conditions favourable for sweet potato production, with better yields being obtained in areas receiving rainfall of over 750 mm per year and with an average ambient temperature of 24 °C. Sweet potatoes will grow from sea level to 2100 metres; at the higher elevations it replaces cassava, which is grown only below 1500 metres in the Highlands. In the Central Province, sweet potatoes are planted at any time during rainfall on flat, deep, friable soil, the plants subsequently forming a dense canopy which reduces the need for weeding. This Province also has the highest population growth rate in Kenya; the concomitant high food demands are causing changes in agricultural practices, with a trend towards replacement of traditional stable cereals by high yielding tuber crops such as potatoes and sweet potatoes. In exceptionally dry seasons, sweet potatoes assume even greater importance.

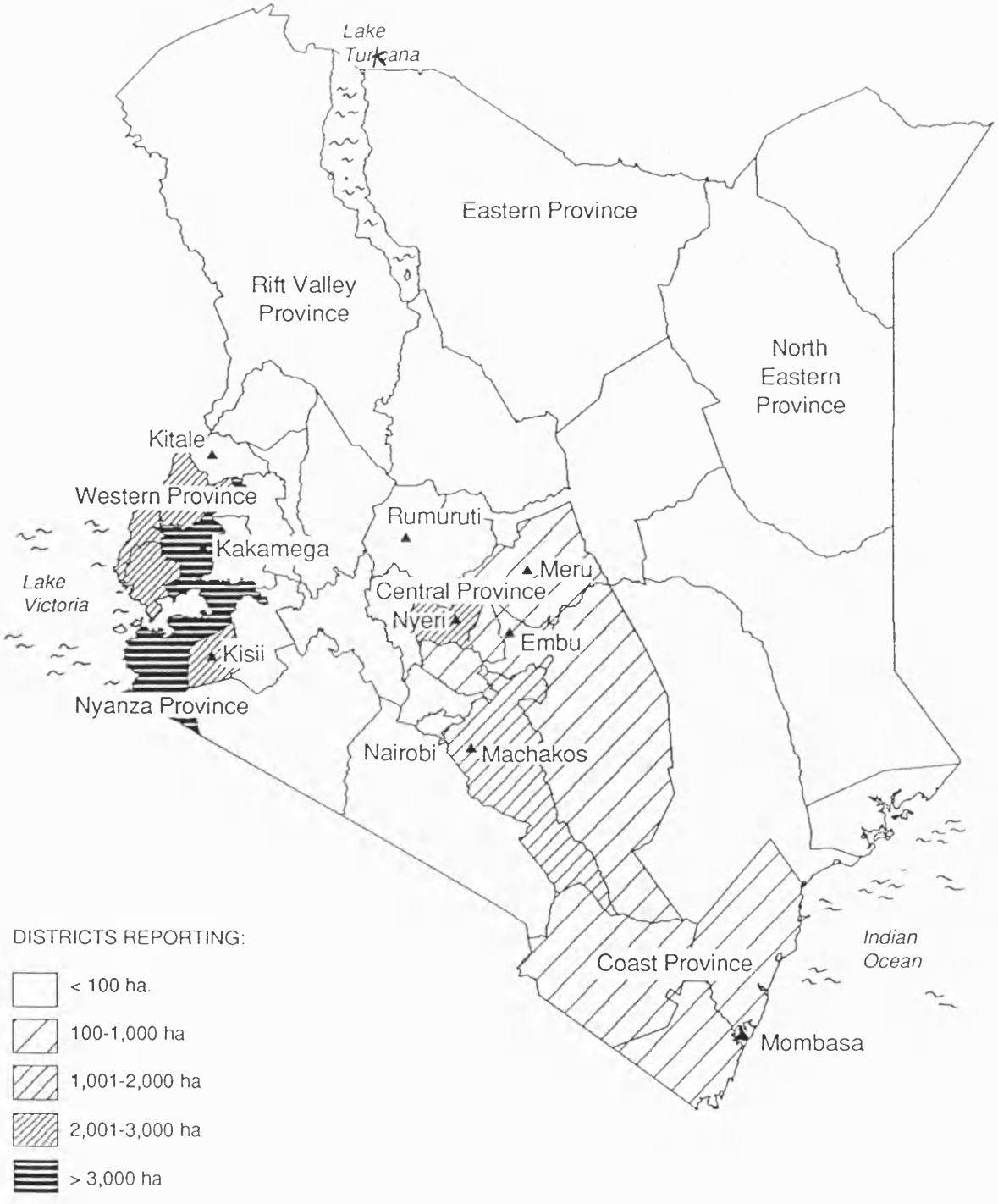
In Western Kenya and marginal areas (Fig. 5) sweet potatoes are usually grown in ridges as annual crops with planting beginning at the commencement of the rainy

season in March to April; crops are also planted at the beginning of the dry season in December in swampy areas to maintain planting stocks for the following season. Many sweet potato cultivars are drought tolerant and will produce acceptable yields even in dry and semi-dry areas provided there is sufficient rain to permit their establishment. Sweet potatoes have, therefore, great potential for providing food during periods of drought.

Because only 1% of arable land in Kenya is at present used for sweet potato production, there is a great need for expansion.

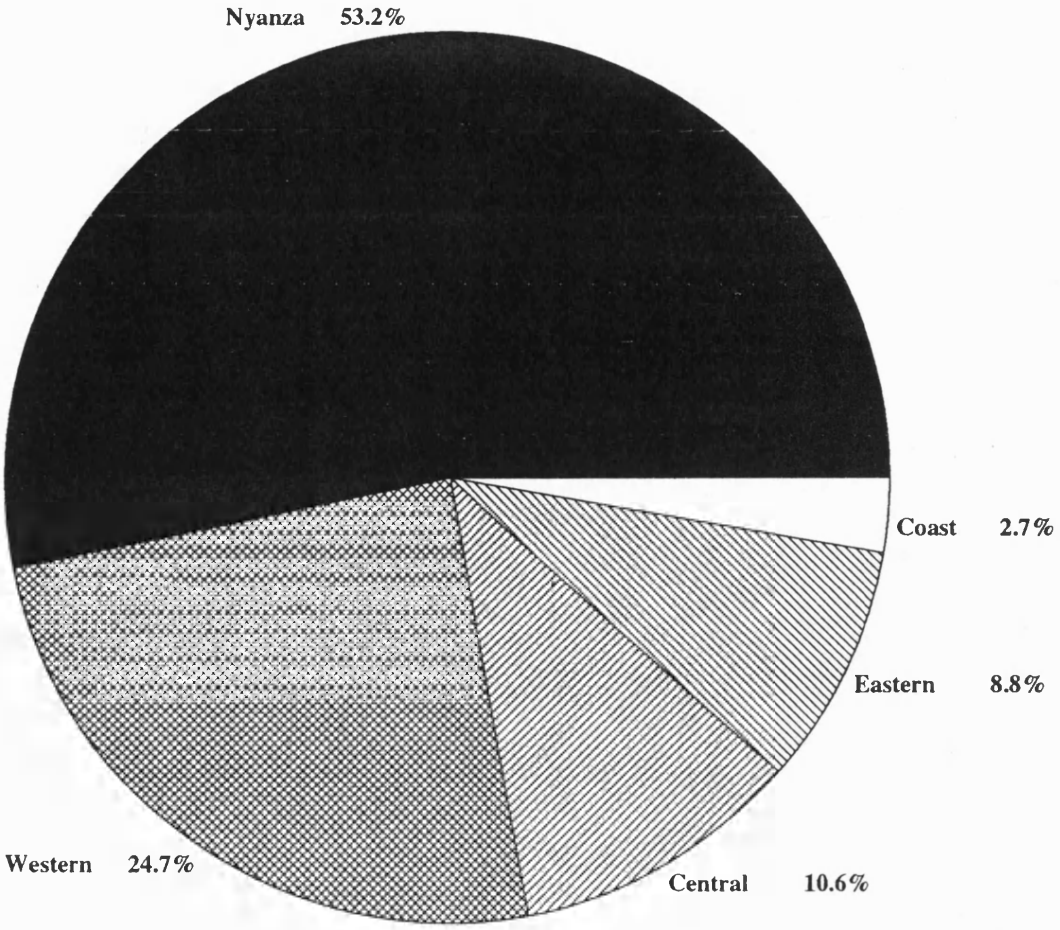
Fig- 5

KENYA: SWEET POTATO PRODUCING AREAS



Source: Kenya, Minisry of agriculture, Provincial Reports for 1985

Fig. 6. PRODUCTION (MEAN AREA) OF SWEET POTATO IN KENYAN PROVINCES (1985-1986)



* Total production area c. 34,000 hectares
(Kenyan Ministry of Agriculture)

1.4 The Origin and Botany of *Ipomoea batatas*

The sweet potato is thought to have originated in Central or South America, probably in the region between the Yucatan Peninsula of Mexico and the mouth of the Orinoco river in Venezuela. For at least 2000 years it has been used for human food in that region and in many islands of the South Pacific. Its dissemination to Polynesia is possibly associated with the extraordinary voyages of early Peruvian or Polynesian explorers and traders. Sweet potatoes are thought to have been first brought to Europe by Christopher Columbus on his return journey from South America, and then taken in the 16th Century by Portuguese explorers to Africa, India and eastern Asia (Onwueme, 1978; Bassett, 1986; Norman *et al.*, 1989).

The sweet potato, although now usually cropped as an annual, is a herbaceous perennial species with creeping or trailing stems (so called "vines") which grow very rapidly and produce a shallow canopy. Genotypes, broadly grouped into bush, intermediate and vining types, vary greatly in branching pattern, internode length and overall stem length. Leaves may also differ greatly in size, shape (broad and entire to deeply indented) and length of petiole. Different types of roots are produced by the plant. Young, thin adventitious roots arising from internodes are thin, but differentiate into heavily lignified fibrous roots. Roots arising from nodes are thick, and differentiate into either "pencil" roots or storage roots. The later are not lignified, enlarge from repeated divisions of the vascular cambium, have a pentarch or hexarch arrangement of vascular tissue, and have a phellogen that produces a thin periderm at the root surface (Onwueme, 1978).

Sweet potato flowers are complete with a compound superior pistil, five separate stamens attached to the corolla and with petals united into a trumpet or bell-shaped corolla. The corolla is usually white at the margin and pink to purple in the throat. Seeds have a hard seed coat and develop within a capsule. The plants usually set few

viable seeds; many genotypes do not readily flower, others are sterile and most are self-incompatible (Bassett, 1986).

Sweet potato is a hexaploid with 90 chromosomes. As most *Ipomoea* species have 30 chromosomes, it is suggested that *I. batatas* arose from a cross between a tetraploid (possibly *I. trifida*) and a diploid species (Norman *et al.*, 1989).

1.5 Major Constraints to Production of Maximum Yields

Although the production of many major food crops in developing countries has in recent years exceeded population increases, that of sweet potatoes has failed to do so (Fig. 7). The main constraints to increased production are yield losses caused by fungal and virus diseases, pests (especially weevils), lack of cultivars suitable for diverse climatic conditions, and marketing difficulties.

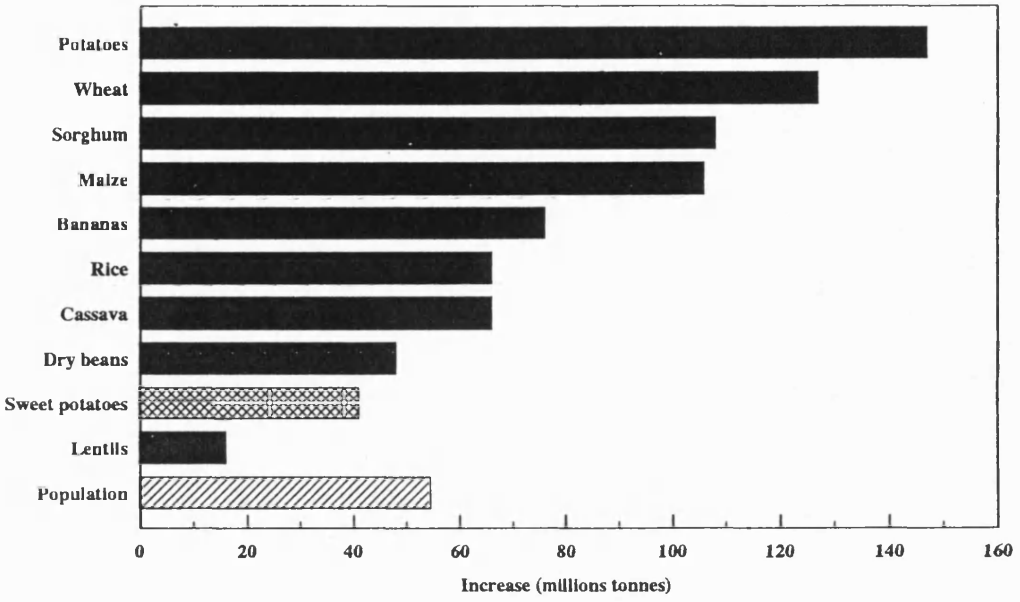
Several viruses have been reported to seriously reduce the yield of sweet potato crops (Sheffield, 1957; Hollings *et al.*, 1976; Hahn, 1979; Loebenestein *et al.*, 1960; Chung, 1981; Mukiibi, 1977; Liao *et al.*, 1979).

Loebenstein *et al.* (1960) reported that sweet potato feathery mottle virus (SPFMV) caused severe yield reductions of sweet potatoes in Israel. Mukiibi (1977) reported yield losses of up to 57% due probably to infection by SPFMV and sweet potato mild mottle virus (SPMMV) in Uganda. In Nigeria, Hahn (1979) reported a tuber yield reduction of 78% caused by sweet potato virus disease complex. In Taiwan, Chung *et al.* (1981) reported yield losses of between 25-56% resulting from mixed infection of SPFMV and sweet potato latent virus (SPLV); however, they recorded that virus infection did not affect the quality (dry matter, protein, and sugars) of fleshy tubers. Production statistics (Table 2) show that African countries have the lowest average yields in the world. Sweet potato yields in East African countries (of c. 5, 6 and 9 t/ha in Uganda, Tanzania and Kenya, respectively; Table 5), although

comparable to that of all African producers (6 t/ha), are far below the world average (14 t/ha, Table 2).

Two viruses have been reported to be prevalent in sweet potatoes in East Africa (Sheffield 1957, Hollings *et al.* 1976), and others were detected during the course of the studies reported in this thesis (see 3.3.1.2). As cuttings for new plantings are taken from existing crops, it is likely that viruses are thereby perpetuated and disseminated. Viruses, therefore, probably contribute significantly to yield losses in all production areas of Kenya.

Fig. 7. INCREASE (%) IN PRODUCTION FROM 1961/65 (AVE) TO 1981 OF 10 IMPORTANT FOOD CROPS IN RELATION TO POPULATION INCREASE IN DEVELOPING COUNTRIES



Includes data for potatoes, sweet potatoes and cassava grown in China (Stone & Gitomer 1989); other statistics from FAO (Anon., 1988)

1.6 Research Objectives

1. Although SPFMV and SPMMV are known to occur in Kenya (Sheffield, 1957; Hollings *et al.*, 1976), no national surveys have been made previously to determine whether any hitherto undescribed virus or virus reported to occur elsewhere (e.g. Moyer and Salazar, 1988) are also present. Comprehensive surveys were made, therefore, to determine the identity, occurrence and distribution of viruses infecting sweet potatoes in the main production areas of Kenya.

   In order to devise rational strategies for the control of virus spread in field-grown, elite virus-free stocks, it was also necessary to study the epidemiology of viruses prevalent in the main sweet potato production area in Kenya. Concurrently, therefore, attempts were made to determine rates of re-infection, effects of distance on the extent of spread and of vector involvement in re-infection.

2. The debilitating effects of virus-infection in vegetatively-propagated field-grown crops are often best minimized by the production and use of virus-free planting stock. There was a need, therefore, to produce virus-free stocks by meristem-tip culture. As this is a relatively inefficient procedure with some plant species, attempts were made to develop more effective methods for producing virus-free plants by culturing meristem-tips on media containing one or more antiviral compounds.

3. With the present great potential of using genetic engineering techniques for plant improvement, attempts were made also to develop *in vitro* methods for plant regeneration from sweet potato cell and callus cultures. Such plant regeneration techniques form an integral part of the majority of genetic engineering procedures.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Plant Materials

Virus-free sweet potato plants of cvs Jasper, Rose Centennial, Brondal and Tib. 9 were provided by Dr A. A. Brunt from a collection at the Horticultural Research International (HRI), Littlehampton, Sussex, England. Sweet potato plants from the same source, but infected with the viruses indicated, were cvs Papota (SPFMV), Jasper (sweet potato ringspot virus; SPRV); and Rose Centennial (SPRV), and accessions numbered 157 (SPFMV, from Fiji), 512 (SPMMV, from Burundi), HIB 687 (SPFMV, from Papua New Guinea) and KS 589 (SPMMV from Kenya). All infected plants were imported and maintained under a licence issued by the British Ministry of Agriculture, Fisheries and Food.

The virus-free cvs Brondal, Rose Centennial and Tib. 9 were maintained in different greenhouses but under the same conditions.

Seedlings of *Ipomoea setosa* and a wide range of indicator plants species were raised and grown in insect-proof greenhouse.

2.2 Plant Growth and Maintenance in the Greenhouse

Sweet potato cuttings 20 to 30 cm long were planted in 9 inch diameter capacity plastic pots containing Fisons C2 compost enriched with 5 g/l Fisons 140 fertilizer (Fisons NP/C) and watered twice daily. The greenhouse was maintained at 27 °C, natural light being supplemented artificially with GEC solar colour HPS 400 W lamps, for a photoperiod of 16 hours (30 $\mu Mm^{-2}s^{-1}$PAR). Pests were controlled by spraying plants with Nicotine (Synchemical), Pynosect 30 (Mitchel Cotts) and Malathion (Farm Protection) every two weeks (alternating the pesticides). The plants

were pruned heavily every two months both to control insects and to encourage the growth of new shoots. Cvs Papota, 157, and HIB 687 infected with SPFMV were maintained together in one chamber of the greenhouse. Cvs Jasper and Rose Centennial (RC) were maintained in a growth chamber (Satchwell Duotronic) under environmental conditions similar to those in the greenhouse, except that lighting was provided by warm white fluorescent tubes (GEC 58W) and incandescent bulbs. These latter plants provided particularly good sources of explants for cell and tissue culture work.

2.3 General Culture Media Preparation

The commercially prepared Murashige and Skoog (MS) basal medium (Flow Laboratories Ltd.), the chemical composition of which is given in Table 6, was used throughout these studies. MS basal medium was supplemented according to culture requirement as described in Table 7 using analytical grade chemicals (Sigma Co. Ltd.). Micro-quantities were measured using an analytical balance (Sartorius), but macro-quantities were measured with an ordinary balance (Mettler); all were added to a one litre plastic beaker containing distilled water (Fisons Fi-streem Water Still), with constant stirring using a magnetic "flea" and stirrer (Gallenkamp). The media were adjusted to pH 5.6 (Orion Research Digital) with 0.1 N NaOH or 0.1 N HCl before the final volume was adjusted. The media were distributed in 1 litre Duran bottles; for solid media, 8 g/l of agar (Oxoid Agar No. 3) was added before sterilization with a bench top autoclave for 15 minutes at 15 lb pressure. The media were then placed in a waterbath 50 °C (Jencons Scientific Ltd) and allowed to cool for 15 minutes before the addition of filter-sterilized chemical supplements or

Table 6. COMPOSITION OF THE MURASHIGE AND SKOOG (1962) MEDIUM

		Concentrations	
		mg/l	μm
Macro elements	NH_4NO_3	1650	20.61
	KNO_3	1900	18.80
	$CaCl_2.2H_2O$	440	3.00
	$MgSO_4.7H_2O$	370	1.50
	KH_2PO_4	170	1.25
	Na_2EDTA	37.3	
	$FeCl_3$	16.2	
Micro elements	H_3BO_3	6.2	100.0
	$MnSO_4.4H_2O$	22.3	100.0
	$ZnSO_4.7H_2O$	8.6	30.0
	KI	0.83	5.0
	$NaMo_4.2H_2O$	0.25	1.0
	$CuSO_4.5H_2O$	0.025	0.1
	$CoCl_2.6H_2O$	0.025	0.1
Organic components	Glycine	2.0	
	Nicotinic acid	0.5	
	Pyridoxine HCl	0.5	
	Thiamine HCl	0.1	
	M-Inositol	100.0	
	Sucrose	30,000.0	

Table 7. COMPOSITION OF INTERNATIONAL POTATO CENTER (CIP) MEDIA FOR SWEET POTATO *In vitro* CULTURES

	Medium (pH 5.6) for		
	Meristem Establishment (Stage I)	Meristem/shoot -tip Transfer (Stage II)	Propagation (Stage III)
	mg/l	mg/l	mg/l
Calcium pantothenate	2	2	2
Gibberellic acid	20	15	10
Ascorbic acid	100	100	200
Calcium nitrate	100	100	-
Putrescine HCl	20	20	20
L-Arginine HCl	100	-	100
Kinetin	0.4	-	-
Sucrose	50 g/l	50 g/l	30 g/l
Agar	7 g/l	7 g/l	8 g/l
Murashige/Skoog salts or Gamborg's B-5 salts	1/2 strength Minimal organics	1/2 strength Minimal organics	Full strength Minimal organics

2.4 General Culture Techniques and Incubation

Young stem pieces of sweet potatoes with several lateral buds were cut with scissors from greenhouse-grown plants. All the leaves were removed and the stems were then cut into smaller pieces, each of which had two lateral buds. The pieces of stem were surface sterilized by washing for 3 min. in 70 % EtOH and then for 10-15 min. in 1.5% sodium hypochlorite containing a few drops of the wetting agent Tween 20 (BDH Co. Ltd.). Finally, they were rinsed four times in sterile distilled water. These manipulations were made in Laminar flow cabinet, and were followed by the excision of cultures using sterile surgical blades (Swann Morton Ltd) and forceps, and their transfer to the prepared medium. The cultures were then incubated either in a growth incubator (Gallenkamp & Co Ltd.) or a growth room maintained at 25$\mp$1 °C and with a photoperiod of 16 hours (30 $\mu M m^{-2} s^{-1}$PAR). Lighting was provided by warm white fluorescent Lamps (GEC, SK).

2.5 Virus Detection and Identification

Viruses infecting sweet potatoes were initially detected by graft inoculating healthy *Ipomoea setosa* with small pieces of sweet potato stems as described by Moyer *et al.* (1989). Attempts were made also to transmit viruses mechanically from graft-infected *l. setosa* or, less frequently, from naturally-infected sweet potatoes to a range of virus indicator plant species which usually included *Nicotiana clevelandii, N. benthamiana, N. tabacum, N. qlutinosa, N. debneyi, Chenopodium guinoa* and *Vigna unguiculata.*

Viruses were specifically identified by serological techniques including enzyme-linked immunosorbent assay (ELISA) and immunosorbent electron microscopy (ISEM).

The diagnostic antisera, all supplied from the reference collection at HR1 Littlehampton were to the following viruses:- SPFMV, SPMMV, SPRSV, SPC-LV, SPLV, SPCSV and CMV.

2.5.1 Transmission to Experimental Hosts

2.5.1.1 Graft-inoculation

Because of the high content of inhibitors of virus infection and/or virus inactivators in *I. batatas,* viruses infecting sweet potatoes were usually graft-transmitted to *I. setosa* before being studied further. In early studies, pieces of infected petiole or stem were inserted into slots cut in the base of young *I. setosa* seedlings, and the cut area was then covered with grafting tape. Latterly, a procedure recommended by IBPGR and FAO, in which stem pieces with 2 or 3 nodes were cleft-grafted to *I. setosa* (Moyer *et al.*, 1989), was used.

2.5.1.2 Mechanical-inoculation

Viruses were transmitted manually from infected to healthy herbaceous hosts by grinding infected leaf tissue (1 g/10 ml) in 0.067 M phosphate buffer at pH 7.6. When attempting the transmission of viruses from sweet potato, either 0.5% (w/v) sodium sulphite or 0.05 M sodium diethyldithio carbamate was added to the buffer to minimise the enzymatic oxidation of polyphenolic materials to potent virus inactivators. After the addition of the abrasive Celite 545, the inoculum was rubbed with the forefinger onto the leaves of plants which, to increase their susceptibility (Bawden *et al.*, 1947 & Bawden *et al.*, 1948), had been kept in the dark for the previous 18-24 hours. Inoculated leaves were then rinsed with water to avoid scorching.

2.5.2 Electron Microscopy

Specimens were negatively stained with either 2% (w/v) neutral phosphotungstate or 2% (w/v) uranyl acetate at pH 3.5-3.8, and then examined in a Jeol 100S electron microscope operating at 80kV. The dimensions of particles were estimated by using catalase crystals (lattice spacing 8.7 nm) as an internal calibration standard (Wrigley, 1968).

2.5.3 Immunosorbent Electron Microscopy (ISEM)

ISEM, both "trapping" and "decoration", was performed essentially as described by Roberts and Harrison (1979). (i) "Trapping". The carbon support film on electron microscope grids was coated with virus-specific antibody antiserum by floating grids for 1 hr at room temperature (c. 18 °C) on 20 µl drops of antiserum diluted to 1/1,000 in 0.067 M Sorensen's phosphate buffer at pH 7.6, contained in wells of a Teflon plate. The grids were then removed, drained by briefly touching the edge of each to filter paper and then washed by floating for 5 min in each of three wells containing 20 µl drops of 0.067 M phosphate buffer at pH 7.6. After washing, the antibody coated grids were drained, placed for 1 hr on 20 µl drops of virus containing extracts, and then negatively stained with potassium phosphotungstate or uranyl acetate (2.5.2).
(ii) "Decoration": When attempting to "decorate" trapped particles, grids removed from drops of virus-containing extracts were washed, placed for 30 min. at 18 °C on 20 µl drops of antiserum diluted to 1/50 in 0.067 M phosphate buffer at pH 7.6, washed, drained and negatively stained as described above.

2.5.4 Enzyme-linked Immunosorbent Assay (ELISA)

Double antibody sandwich-ELISA:

The method mainly used was, with minor modifications, the double antibody sandwich ELISA (DAS-ELISA) of Clark and Adams (1977). The buffers used in the assays were:

Coating buffer (0.05M carbonate buffer at pH 9.6)

0.015 M sodium carbonate (Na_2CO_3)

0.035M sodium hydrogen carbonate ($NaHCO_3$)

0.003M sodium azide (NaN_3)

Phosphate buffered saline (PBS) at pH 7.4

0.137 M sodium chloride (NaCl)

0.0015M potassium dihydrogen orthophosphate (KH_2PO_4)

0.0081 M dissodium orthophosphate dihydrate ($Na_2HPO_4.2H_2O$)

0.0027 M potasium chloride (KCl)

0.003 M sodium azide (NaN_3)

PBS Tween (PBS-T)

PBS (as above)

0.05% Tween 20 (polyoxyethylene sorbitan monolaurate)

Extraction buffers

PBS Tween (as above)

2% polyvinylpyrrolidone

0.2% ovalbumin

Substrate buffers

(i) for alkaline phosphatase (pH 9.8)

9.7% diethanolamine in distilled water

0.003 M NaN_3

(ii) for horseradish peroxidase

0.1 M sodium acetate (adjusted to pH 6.0 with citric acid)

20 mg of 3,3', 5,5' tetramethylbenzidine in 1 ml of dimethyl sulphoxide

0.006% of the final volume of hydrogen peroxide (added immediately prior to use)

The diagnostic antisera were from the reference collection at HR1/Littlehampton (see 2.5), but another to SPFMV was also kindly supplied by Dr J. Moyer. The antisera used were:- SPFMV, SPLV, SPMMV, SPCSV, SPC-LV, SPRSV, and CMV. The gamma-globulins of each were separated from the sera as described by either O'Donnel *et al.* (1982) or Clark and Adams (1977); when necessary, the antisera were first absorbed with healthy sap to remove antibodies to normal plant proteins (mostly ribulose biphosphate carboxylase). Gamma-globulin-alkaline phosphatase conjugates were prepared to each antiserum as described by Clark and Adams (1977).

The gamma-globulins to each virus were diluted to 1 µg/ml in coating buffer and 200 µl were added to each well of a microtitre plate (excluding the outermost wells which sometimes give spurious results). The plates were included in a plastic bag for 2-4 hr at 37 °C. The well contents were then discarded and the wells washed thoroughly three times (for 3 min. on each occasion) with PBS containing 0.05% Tween 20.

Leaf tissue to be tested was ground (1 g / 20 ml) in extraction buffer; 200 µl of each sample was added to duplicate wells and, after incubation for 2 hr at 37 °C, the well contents were discarded and the plates washed and dried as previously described.

The gamma-globulin-enzyme conjugate was diluted to 1/1,000 with extraction

buffer and 100 µl incubated in each well for 2 hr at 37 °C. The plates were then washed and dried as described previously.

The substrate, p-nitrophenyl phosphate, was dissolved in substrate buffer to a final concentration of 0.67 mg/ml and 150 µl added to each well. The solution in wells in which positive reactions occurred usually became yellow in 30-60 min. at which time the reaction was stopped by the addition of 50 µl/well of 3N NaOH. The optical density of the contents of each well was measured at 450 nm wavelength immediately using a Titertek Multistan Plus Mark II plate reader.

2.5.5 Enzyme-Linked Immunosorbent Assay on Nitrocellulose Membrane (NCM-ELISA)

The NCM-ELISA technique used throughout these studies was, with only slight modifications, that described by Lizarraga and Fernandez-Northcote (1989).

Sample extraction: Leaf samples from naturally infected sweet potatoes or experimental hosts (especially *I. setosa* and *Nicotiana* spp.) were ground (1 g/10 ml) in an extractant consisting of tris-buffered saline buffer at pH 7.5 containing 0.1 M diethyldithio carbamate and 0.02% sodium azide. The samples were ground by rolling a bottle over sealed plastic bags, each of which contained one sample and the extractant. Approximately 0.8 ml of each extract was transferred to an Eppendorf tube, 0.4 ml of chloroform added and, after thoroughly shaking, the mixture was clarified by centrifugation for 2 min. at c. 9,500 g.

A 200 µl portion of each clarified extract was diluted with 800 µl of extractant (final sap dilution 1/50) and stored overnight (16-18 hr) at -20 °C.

Sample Application: Sheets (60x30 cm) of nitrocellulose membrane (Schleicher and Schuell, Inc., 0.4 µm pore size) were marked into 1 cm squares with a black ball point pen, and then cut into 8x8 cm squares with the bottom right hand corner of

each being marked with ink. The NCM sheets were immersed for 5 min. in distiled water and then for 5 min. in TBS. Whatman No. 4 filter papers were treated similarly and placed on dry untreated filter paper overlying dry paper towels. The NCM was placed on the TBS-treated filter paper and allowed to dry for 2 min. Ten µl of each sample, and of appropriate positive and negative controls, were applied with a micropipette to each 1 cm square. The NCM was then transferred to a dry Whatman No. 4 filter paper, the applied samples allowed to dry overnight and, after wrapping in paper tissue, the NCMs were stored temporarily in a desiccator.

Antigen detection: The NCM sheets were each placed for 1 hr in 16 ml of "blocking" buffer (TBS containing 3% bovine serum albumin), washed briefly in TBS and then shaken overnight (c. 18 hr) on an orbital shaker (operating at 50 rpm) after enclosing each in a plastic bag with 16 ml "blocking" buffer containing the relevant IgG at an appropriate dilution determined previously. The IgGs to SPFMV SPMMV, SPLV, SPC-LV, SPCSV, SPRSV were used at a dilution of 1/500, but that to CMV was used diluted to 1/2000. The IgGS were separated from the antisera by Dr Fernandez-Northcote.

Before use, IgGs of SPFMV, SPCSV, and SPLV were cross-absorbed with healthy plant proteins as follows; healthy *I. setosa* leaves were ground in "blocking" buffer (1 g/49 ml) with a pestle and mortar, the fluid was then separated from course plant debris by squeezing through cotton gauze, 16 ml was added to the IgG at the appropriate dilution, and then mixture incubated for 1 hr at 37 °C.

Each NCM was shaken with IgG solution overnight in an orbital shaker (operating at 50 rpm), rinsed with TBS containing 0.05% Tween-20 (TTBS), and then washed thoroughly three times with TTBS. Membranes were then incubated for 1 hr with goat anti rabbit IgG-alkaline phosphatase conjugate diluted with TBS containing 1% BSA, and then washed with TTBS. The NCM were then immersed in the dark for

20-60 min. in p-nitroblue tetrazolium solution (NBT) (35 mg in 100 ml of 0.1 M tris at pH 9.5) containing 10 µl of 5-bromo -4- chloro -3- indolyl phosphate (BCIP) (5 mg/100 µl N.N.-dimethylformamide).

Membranes were then washed three times with distilled water, placed on dry filter paper and then dried overnight in the dark. The dry NCMs were mounted on A-4 photocopying paper for subsequent analysis and storage. The spots were bluish purple in positive readings and greenish, or colourless in negative readings.

2.5.6 Double Gel-diffusion Tests

These were made in 0.7% (w/v) ionagar gels mounted on microscope slides essentially as described by Mansi (1958).

2.5.7 Virus Purification

SPFMV and SPMMV were purified from *I. setosa* or *N. benthaniana*, respectively, by the procedure described by Moyer and Cali (1985). SPC-LV was purified from *I. setosa* essentially as described by Brunt (1971) for dahlia mosaic virus (DMV).

CHAPTER 3

STUDIES OF SWEET POTATO VIRUSES

3.1 INTRODUCTION

SPFMV and SPMMV have long been known to occur in Kenya (Sheffield, 1957; Hollings *et al.*, 1976). However, it was unknown if any hitherto undescribed viruses and which, if any, of the viruses reported to infect *I. batatas* elsewhere were also present in cultivars grown in Kenya. Surveys were made, therefore, to determine the occurrence and identity of viruses present in sweet potato cultivars currently grown in the major production areas of the country. After many preliminary tests, NCM-ELISA (2.5.5) was chosen as an effective method for the rapid detection and identification of viruses occurring in naturally-infected *I. batatas* plants or in graft-inoculated indicator species (especially *I. setosa*); DAS-ELISA (2.5.4) and ISEM (2.5.3), however, were also used occasionally.

No information was available on the epidemiology of the viruses infecting sweet potatoes in Kenya, and therefore few data on the seasonal occurrence and identity of known, or potential insect vectors, of the viruses. Preliminary epidemiological studies were made, therefore, so that such information might facilitate the development of appropriate strategies to minimise the re-infection of field-grown elite virus-tested stocks that were being produced concurrently (chapter 4).

The results of the virus surveys and the epidemiological studies are here recorded.

3.1.1 VIRUSES KNOWN TO INFECT SWEET POTATO

3.1.1.1 Viruses with Filamentous Particles

3.1.1.1.1 Sweet Potato Feathery Mottle Virus (SPFMV)

SPFMV is now the accepted name for the virus which was previously also described as sweet potato russet crack, sweet potato chlorotic leafspot, sweet potato internal cork or sweet potato A virus. Past confusion in nomenclature was due mainly to differences in symptoms induced by closely related strains of the virus in different cultivars (Cadena-Hinojosa and Campbell, 1981; Cali and Moyer, 1981).

SPFMV is the most widespread and prevalent of the viruses infecting sweet potatoes, and often occurs together with other viruses world-wide. It is readily transmitted in a non-persistent manner by aphids of which *Myzus persicae* (Stubbs and McLean, 1958; Webb and Larson, 1954; Rankin, 1950; and Sheffield, 1957), *Aphis craccivora* (Moyer and Kennedy 1978), Aphis *gossypii* (Stubbs and Mclean, 1958; Kantack, Martin and Newson, 1958), *Lipaphis erysimi* (Moyer and Kennedy, 1978), and *Macrosiphum euphorbiae* (Hildebrand and Smith, 1988) are known to be important vectors. The virus is not seed-borne (Cadena-Hinojosa and Campbell, 1981) but, like many viruses infecting vegetatively propagated plant species, is disseminated in infected tubers and cuttings.

In addition to its major host, the virus will infect other members of the Convolvulaceae including *I. nil, I. purpurea, I. hederacea, I. wrightii, I. incarnata* and *I. trichocarpa.* In Louisiana (USA), *I. trichocarpa* is a perennial reservoir and *I. hederacea* and *I. wrightii* are annual reservoirs of infection (Clark *et al.*, 1986); elsewhere, however, the importance of uncultivated infected *Ipomoea* spp. or foci of infection has not been determined. Some strains or isolates of SPFMV will also induce local lesions in *Chenopodium amaranticolor* and *C. quinoa* and a few infect *Nicotiana clevelandii* systemically. The virus, however, has failed to infect any of

19 other plant species from 11 families (Brown *et al.*, 1987). SPFMV induces the formation of cytoplasmic intracellular inclusions ("pinwheels") in infected plants (Lawson and Heason, 1971; Lawson *et al.* 1971).

SPFMV is purified with difficulty, mainly because of the propensity of the particles to aggregate *in vitro* (Moyer and Kennedy, 1978; Cali and Moyer, 1981; Moyer and Cali, 1985; Brown *et al.*, 1987). However, yields of at least 20 mg g^{-1} leaf tissue can be obtained from infected *I. setosa* or *I. nil*, possibly the best available propagation hosts of the virus. More recently, a procedure has been developed for its successful purification from sweet potato (Cohen *et al.*, 1988). SPFMV has filamentous particles mostly ca. 850 nm long, which contain SS-RNA of M_r $3.65x10^6$ and coat protein of M_r $36x10^6$ to $38x10^3$ (Moyer and Cali, 1985). These properties indicate that SPFMV is a member of the potyvirus group.

Isolates of the virus from widely scattered geographical locations are serologically closely related, as are pathogenically distinct strains in the USA (Cadena-Hinojosa and Campbell, 1981; Cali and Moyer, 1981). SPFMV, however, has shown no serological relationship to any of 16 other potyviruses (Brown *et al.*, 1987).

The virus can be easily detected and identified by ISEM or ELISA in *I. setosa* and *I. nil* and, under appropriate conditions, also in sweet potatoes.

3.1.1.1.2 Sweet Potato Latent Virus (SPLV)

SPLV, originally designated SPV-N (Liao *et al.*, 1979), has hitherto been reported only in Taiwan. Unlike SPFMV, it is readily sap-transmissible to 14 of 36 species of the Convolvulaceae, Solanaceae and Chenopodiaceae; of these, *N. clevelandii* and *N. benthamiana* are good propagation hosts and *C. amaranticolor* and *C. quinoa* sensitive local lesion assay hosts (Brown *et al.*, 1987). The virus is

neither seedborne nor transmitted by aphids (*M. persicae*) but, like other sweet potato viruses, is disseminated in infected tubers and other vegetative propagules.

SPLV is a little easier to purify than SPFMV. It has filamentous particles mostly 700-750 nm long which contain ss-RNA and a capsid polypeptide of M_r $40x10^3$. The virus is serologically distinct from other filamentous viruses infecting sweet potato, and from 17 distinct potyviruses (Moyer and Kennedy, 1978; Moyer and Cali, 1985).

SPLV is readily detected in some hosts by ISEM and ELISA.

3.1.1.1.3 Sweet Potato Mild Mottle Virus (SPMMV)

This virus was first detected in Kenya by Sheffield (1957), although then described as sweet potato virus B; it was later designated SPMMV and reported to occur also in other East African countries (Hollings, Stone and Bock, 1976). It is readily sap-transmissible to a wide range of herbaceous plant species; it infects 45 of 119 species in 14 of 36 families, of which *N. tabacum* and *N. benthamiana* are convenient-propagation hosts and *C. guinoa* a sensitive local lesion assay host. The virus is not seedborne, but is transmitted by *Bemisia tabaci*. SPMMV has filamentous particles 850-950 nm long, which sediment as a single component with a sedimentation coefficient ($S°20,w$) of 155 S and contain ss-RNA and a capsid polypeptide of M_r $38x10^3$. The virus, however, has yet to be fully characterised.

The virus is detectable by ISEM and ELISA in sweet potatoes and other hosts. It shows no serological relationship to other sweet potato viruses nor to 16 members of the potyvirus group (Hollings *et al.*, 1976), and is the type and sole member of the proposed Ipomovirus group of the proposed Potyviridae family (A.A. Brunt, personal communication).

3.1.1.1.4 Sweet Potato Chlorotic Stunt Virus (SPCSV)

SPCSV is the tentative name for a filamentous virus isolated from sweet potatoes from the Caribbean area which, apparently, differs from others previously reported in sweet potatoes (Brown *et al.*, 1987). The virus was originally isolated from stunted plants with chlorotic leaves. It is readily sap-transmissible to a wide range of herbaceous species of which *N. benthamiana* is a good propagation host.

The virus is not transmitted through seed nor by aphids. It is easily purified and, like SPFM and SPMMV, has filamentous particles mostly 850-950 nm long, which contain ss-RNA and a single capsid polypeptide. Like other viruses, SPCSV can be detected and identified by ISEM and ELISA. The virus is now being further characterized (personal communication A.A. Brunt).

3.1.1.1.5 Sweet Potato Yellow Dwarf Virus (SPYDV)

SPYDV was recently described in Taiwan by Green (1989). The symptoms induced by this virus in sweet potato plants, which are favoured by poor soil fertility and low temperature, include leaf mottling and chlorosis and plant dwarfing. The tubers of infected plants are also poorly developed and often unmarketable. The virus frequently occurs in sweet potatoes together with SPFMV. In *I. setosa* it causes stunting, general leaf chlorosis, small distinct chlorotic spots, and veinal chlorosis. The host range of SPYDV includes other species of the Convolvulaceae, *Chenopodium* spp, *Gomphrena globosa, Sesamum orientale, Datura stramonium* and *Cassia occidentalis.*

SPYDV, like SPFMV, has flexuous filamentous particles 750 nm long; they have a capsid protein of M_r $33x10^3$, but its nucleic acid has yet to be characterized. The virus is mechanically transmissible and is transmitted naturally by *Bemisia tabaci*. It is detectable in infected hosts by ISEM and ELISA.

3.1.1.1.6 Sweet Potato Vein Mosaic Virus (SPVMV)

This so-called virus has been reported only from Argentina (Nome, 1973). It has flexuous filamentous particles which, being 761 nm long, are significantly shorter than those of SPFMV and SPMMV (Nome *et al.*, 1974). The virus is transmitted in the non-persistent manner by aphids. Unfortunately, the original culture and a homologous antiserum are not available, so comparisons with other sweet potato viruses have not been possible (Moyer *et al.*, 1989).

3.1.1.2 Viruses With Isometric Particles

3.1.1.2.1 Sweet Potato Caulimo-Like Virus (SPC-LV)

The virus was first detected in sweet potatoes originating from Puerto Rico (O. M. Stone and A.A. Brunt, personal communication). It has since been detected in complex with SPFMV in sweet potatoes from Papua New Guinea, New Zealand, Solomon Islands and Madeira. The vectors of the virus are unknown. In some leaves of graft-inoculated *I. setosa* seedlings it induces isolated interveinal chlorotic spotting and occasional chlorotic veinal flecks (Atkey and Brunt, 1987). Purified preparations contain isometric particles c. 50 nm in diameter which contain a major polypeptide of M_r $42-44x10^3$ and ds-DNA. The virus is moderately antigenic, but comparative serological tests have shown it to be serologically unrelated to cauliflower mosaic, dahlia mosaic and carnation etched ring caulimoviruses and a similar virus (soybean chlorotic mottle virus) infecting soybean in Thailand (Iwaki *et al.*, 1984).

Virus particles and characteristic intracellular inclusions induced by the virus are readily detected in the cytoplasm of epidermal and vascular parenchyma cells of infected plants (Atkey and Brunt, 1987). Ultrastructural studies have shown that infected vascular perenchyma cells containing inclusions sometimes protrude into,

and so cause occlusion of adjacent zylem vessels which results in wilting and premature abscission of infected leaves.

The virus is detectable by ISEM and ELISA.

3.1.1.2.2 Sweet Potato Ringspot Virus (SPRSV)

SPRSV is the provisional designation for an apparently hitherto undescribed virus isolated from cv. Wanmum from Papua New Guinea. It was first detected by the unusually mild leaf chlorosis that is induced in graft-inoculated *I. setosa* test plants (Brown *et al.*, 1987). It is sap-transmissible to a moderately wide range of dicotyledonous indicator species, of which *N. benthamiana* and *N. megalosiphon* are the best available propagation hosts. The virus can be purified without difficulty, and has isometric particles c. 28 nm in diameter which sediment as three components with sedimentation coefficients of 60, 90, and 132 S and contain a single polypeptide, M_r $56x10^3$; the "middle" (90S) and "bottom" (132S) component particles contain ss-RNAS, respectively, of c. 6,670 and c. 8,448 nucleotides (J. D. Brown and A. A. Brunt, personal communication). Although the virus has some properties typical of nepoviruses, it is serologically unrelated to any of 12 members of the nepovirus group. The virus and its components are now being further characterised (personal communication A. A. Brunt). It is detectable by ISEM and ELISA.

3.1.1.2.3 Cucumber Mosaic Virus (CMV)

CMV is geographically very widespread and has an extensive natural host range; although it is reported to infect more than 190 species belonging to 40 families, its natural occurrence in sweet potatoes was only recently reported in Israel (Cohen *et al.*, 1988). CMV was found to infect only sweet potato plants already containing SPFMV; together they cause stunting, chlorosis, and yellowing. CMV is transmitted

mechanically, by seed in some hosts, and by many aphid species in non-persistent manner.

CMV has been well characterised and is readily detected and identified by serological techniques (Francki *et al.*, 1979 and Kaper *et al.*, 1981).

3.1.1.2.4 Sweet Potato Leaf Curl Virus (SPLCV)

SPLCV was first found in sweet potatoes in Taiwan (Liao *et al.*, 1979) but it has since been recorded also in Japan (Yamashita *et al.*, 1984). The virus, which induces upcurling and vein swelling of young leaves, is transmitted from infected to healthy sweet potatoes by *Bemisia tabaci* (Chung *et al.*, 1985). The virus has bacilliform particles measuring *c.* 130x30 nm which occur *in vivo* in the cytoplasm of phloem cells. The virus is a possible member of the proposed "badnavirus" group (B.E.L. Lockhart and R. Hull, personal communication).

3.1.1.3 Sweet Potato Virus-Like Agent (SP-VLA)

An uncharacterised virus-like agent which is transmitted by *Bemisia tabaci* has been reported in Nigeria, Israel and Taiwan (Hahn, 1979; Cohen *et al.*, 1990). The agent is not sap-transmissible and alone induces very mild or more usually no symptoms in sweet potatoes or *I. setosa*. It interacts synergistically with SPFMV in both hosts to induce very severe diseases, that of sweet potatoes in Nigeria being designated sweet potato virus disease (Hahn, 1979). The virus-like agent is detected in Nigeria by graft-inoculating the suspect specimen to a susceptible sweet potato genotype (TIB 8, sub-clone 9A or cv. Porto Rico) infected with SPFMV; the development of the characteristically severe symptoms of the complex indicating the presence of the virus-like agent.

Two diseases resembling Nigerian sweet potato virus disease, designated

Georgia mosaic and yellow dwarf, have been reported in the USA (Girardeau, 1958). It is, however, unknown whether the whitefly-transmitted agent associated with these diseases is the same as that occurring in Nigeria, Israel and Taiwan.

Another uncharacterised whitefly-transmitted virus-like agent occurs in sweet potatoes in Nigeria; this does not react synergistically with SPFMV and alone induces leaf-curling in sweet potato (Cohen *et al.*, 1990).

3.1.1.4 Other Viruses Isolated From Sweet Potato

Two viruses with extensive natural host ranges have been isolated from naturally-infected sweet potatoes. Tobacco mosaic virus has been detected only in USA (Elmer, 1960) and tobacco streak virus in sweet potatoes originating from Guatemala (Moyer and Foster, 1986). Tobacco ringspot virus has been transmitted experimentally to several *Ipomoea* spp., but it has not been found to occur naturally in these species (Martin, 1962).

3.1.1.5 Uncharacterised Sweet Potato Viruses

As a result of recent intensification of research, some as yet uncharacterised viruses have been found infecting sweet potatoes. These include sweet potato virus II and sweet potato mosaic virus in Taiwan, isolates C2-C6 in Peru and a newly recognized virus in Puerto Rico (Moyer *et al.*, 1989). Because these viruses have yet to be characterised, comparisons with those detected in Kenya were not possible.

3.2 MATERIALS AND METHODS

3.2.1 Re-infection of Virus-free Sweet Potatoes

3.2.1.1 Experimental sites

Re-infection experiments and insect monitoring were performed at the KARI stations of NARC-Muguga, NARC-Katumani, RRC-Embu, and RRC-Kakamega. The stations were selected because they are located in major sweet potato growing regions or potentially important growing areas and they are in different ecological zones in which different farming systems are used and local staff have research interests in sweet potatoes and could thus provide necessary support. The altitude, ecological zone, rainfall and distribution, temperature, evapotranspiration, soils, and the main crops of the four sites are summarised in Appendix 1. At Muguga (Central Province) and Embu (wet zone of Eastern Province), sweet potato is grown as a perennial crop and may remain in the ground for two to six years with tubers being harvested for food and animal feed as required. Crops are therefore planted in deep, flat, friable well drained soils. At Katumani (dry, arid and semi-arid regions of Eastern Province) and Kakamega (Western and South Nyanza Provinces), sweet potato is grown as an annual crop, which is planted on mounds or ridges and harvested 4 to 6 months later. In Katumani, annual cropping alleviates problems of drought and weevil infestation, and in Kakamega it permits increased yields by alleviating weevil problems.

3.2.1.2 Planting materials

As virus-free planting materials of Kenyan genotypes were unavailable, it was necessary to use sweet potato germplasm originating as tissue cultures from IITA, Nigeria and subsequently maintained at PQS, KARI. Unfortunately insufficient supplies of these were available for comparable replicated experiments at all four

locations, even after further greenhouse multiplication, due to the limited time that was available before commencement of the long rains of the planting season in March/April, 1989.

In February 1989, about 10 cuttings of each of the 12 IITA sweet potato varieties were obtained from Dr. Okioga, the director Plant Quarantine Station (PQS). These plants were further multiplied by establishing 2-nodal cuttings of each in Muguga greenhouse soil mixture contained in 2 litre black plastic bags which were watered daily and maintained in insect free greenhouses. For the re-infection experiments, each variety 108 cuttings of each variety were needed.

Table 8. GENOTYPES USED IN RE-INFECTION EXPERIMENTS

Location	VAR. 1	VAR. 2	VAR. 3
Embu	22297	22294	22290
Muguga	21143	22280	13648
Kakamega	22293	22298	22299
Katumani	22279	22281	22302

These IITA varieties, which had been imported for sweet potato improvement programmes at KARI stations, had not previously been fully field tested or released so their field performance in Kenya, therefore, was unknown.

3.2.1.3 Experimental design

The same experimental design was used for the re-infection experiments at all four locations. Each experimental plot (24x13 metres) consisted of three randomised blocks, each planted with 13 rows (9 of the three cvs and 4 of infectors) 75 cm

apart, and with each row containing 12 plants 50 cm apart. These are the most commonly plant spacings used in most regions in Kenya. Planting in all locations was done between the third week of March to the second week of April. In Muguga and Embu, the sweet potato cuttings were planted on flat, deep friable soils, but in Katumani and Kakamega they were planted in ridges in accordance with local practices. The plant spacings were made using a measuring tape, and sisal ropes and wooden sticks to mark rows and plant positions. Planting holes, 15 cm diameter by 20 cm deep, were dug on each of the 12 positions of each row, either on flat ground or ridges depending on the experimental locations. Rows 1, 5, 9 and 13 were planted with infector stocks c. 20 cm long, which had been picked freshly from symptom expressing sweet potato vines growing within that location. Other planting materials were transported in plastic bags to the experimental plots; the cuttings, which were then c. 20 cm long with well established roots, were gently removed with the intact soil from the plastic bags lowered into the hole and then covered with soil. Because it was the rainy season, it was unnecessary to water the plants. Using waxed paper labels tied to sticks 2 feet above ground level, each row was labelled with variety and other details. Clear labelling was especially important as different rows served different experimental purposes. For example rows 2, 4, 6, 8, 10 and 12 were guard rows, and only rows 3, 7 and 11 contained plants to be assayed. Each assay row per block was of a different variety, and was replicated three times. Plots were regularly weeded to facilitate plant establishment. After the plants were established, they were cut back to c. 28 cm every three months to prevent the vines from overlapping and individual plants from loosing their identity. The pruning was done aseptically, with the knives being disinfected with methylated spirit before trimming each plant. Plants were carefully checked for colonising aphids and whiteflies before pruning. The plots

were located at least 100 metres from the nearest sweet potato field hopefully to ensure that the planted infectors were the only sources of infection. The experimental plots were maintained from March 1989 to November 1990.

3.2.1.4 Virus Identification

Tests for the occurrence of viruses were made to coincide with the end of the sweet potato growing season especially in areas where it is grown as an annual crop. For example, in Kakamega the early to late maturing varieties take between 4 to 6 months to mature and September is the main harvesting period. The second assays done in May 1990 and for areas where sweet potato is grown as an annual, this coincided with the harvest time for the sweet potato crop planted during the short-rains of October to November 1989, a season that is especially important in semi-arid areas like Katumani. It was also important at all locations to have an assay that would show the viruses acquired by the test plants within a one year period. In order to do this, the vines were heavily pruned to leave only one small leafless shoot. Fresh leaves from shoots sprouting from the tubers were collected and assayed for infection. The third and final assays were done in November 1990; they were not timed for any specific sweet potato season, but was important because it indicated the extent of virus spread that had occurred within about one and half years. The final assays were not possible at Kakamega because the plants had been totally destroyed by weevils and virus infection by the end of first year. No insecticidal sprays were used near the experimental areas to avoid harming the virus vectors. Assays were made by picking about 2-3 leaves (weighing in total about 1 gram) from the second to the eleventh plant of the assay row and storing them temporarily

in a labelled sample plastic bag. The samples were assayed for infection by NCM-ELISA at Muguga as described previously in detail (2.5.5). At the time of the final assays, some weeds were also checked for virus infection.

3.2.2 Effects of Distance and Prevailing Southerly Wind on Spread of Sweet Potato Viruses

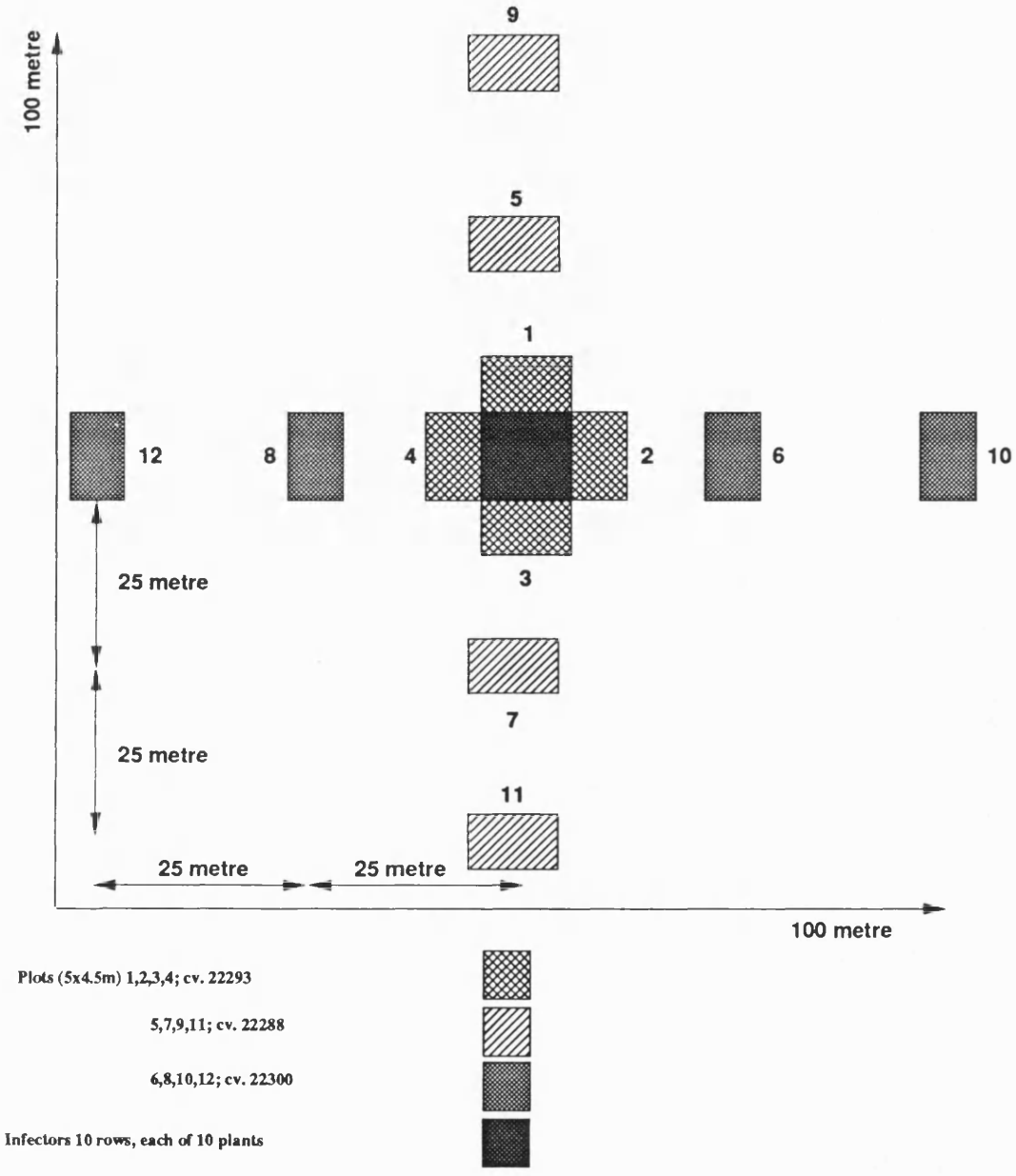
3.2.2.1 Experimental Site, Materials and Design

This experiment was done only at Muguga (Appendix 1) because the large experimental plot (100 m^2) required was only available in this location. The three varieties used for this experiment (22293, 22288 and 22300) were propagated in the greenhouse because about 240 plants of each variety were needed for the experiment. The infectors were obtained from Muguga.

The detailed experimental design is shown in Fig 8. The experimental area consisted of a 100 metre square plot containing a large central plot of 5x5 metres planted with infectors and 5 m by 4.5 m surrounding blocks planted with healthy plants. The spacing between plants of 50 cm and between rows of 75 cm were similar to those of the re-infection experiments, but 10 instead of 12 plants were planted per row . The surrounding plots contained 6 rows each of 10 plants (a total of 60 plants).

The experimental area was planted in the third week of April 1989 in the manner described previously. The plots were regularly weeded to facilitate early establishment of plants, which were later pruned at three monthly intervals as described before. This experiment was located in an isolated area surrounded by Kikuyu grass and nappiergrass pastures and was about one kilometre away from the nearest sweet potato field.

Fig. 8. EXPERIMENTAL LAYOUT OF THE FIELD TRIAL AT MUGUGA TO STUDY THE EFFECTS OF DISTANCE AND PREVAILING WIND ON VIRUS SPREAD



3.2.2.2 Virus Assay

The virus assays were done at intervals of about six months from the time of planting (i.e., in November 1989, May 1990 and November 1990). The assays were not timed to coincide with any particular season because Muguga is in an area in which sweet potatoes are planted as a perennial crop at any time there are rains. They were considered to be reasonable intervals of time to check for re-infection at various distances and directions from foci of infection. The plants assayed included the twenty four plants at the central part of the plot; samples (each of 2-3 leaves) were obtained from plants of the second to fifth rows, and from the third to the eighth plants inclusive (4 rows x 6 plants = 24 plants); the other plants acted as guard rows. Viruses were assayed by NCM-ELISA as described previously (section 2.5.5).

3.2.3 Monitoring of Aphids and Whiteflies

To facilitate the interpretation of the re-infection experiments (see 3.2.1), aphids and whiteflies were trapped at all four experimental sites (Tables 19, 20 & 21) from December 1989 to November 1990. The species trapped were identified and their populations during this period were determined.

A yellow water trap similar in design to that originally described by Moericke (1951) was placed 25 cm above soil level at the centre of each experimental area. One whitefly sticky trap was also located at each site; this was placed 2 meters from the edge of the experimental area, being located upwind and in line with the direction of the prevailing wind.

The Moericke trap, including slight design modifications by CIP, consisted of a galvanised iron tray 10 cm deep and 60 cm square with on one side, a mesh-covered overflow aperture (2.5 cm diameter) 7 cm from the bottom of the tray. The trays

were filled to the overflow level with water containing 0.1% detergent (Teepol). The overflow was especially necessary during periods of heavy rainfall, during which loss of trapped insects from excess water was prevented by the fine mesh cover.

Catches were collected from the traps three times weekly by carefully transferring insects with a fine camel's hair brush to bijou bottles containing 70% (v/v) ethanol; the three weekly collections were aggregated. Every week the trays were emptied, cleaned of debris and refilled with fresh water containing 0.1% detergent (Teepol) to break surface tension and thus facilitate drowning of insects.

Catches of aphids were separated from those of whiteflies in the laboratory by Mr. Robert Opondo using a binocular microscope and fine brushes. Aphid species were kindly identified initially by Dr. Autrique and latterly by Mr. Perreaux (Institute de Sciences Agronomique du Burundi at Bujumbura), and whiteflies by Dr. Lincoln Fishpool (Natural Resources Institute, Chatham, England).

In addition to the use of insect traps, experimental plants were inspected closely at monthly intervals for colonisation by insects.

The sticky trap was basically similar to that originally described by Doncaster and Gregory (1949), but incorporated design modifications by CIP (Dr. Fernandez-Northcote, personal communication). Each trap, which was firmly embedded in the soil, consisted of a polyvinyl chloride tube measuring 3x0.1 metres, and was painted with five yellow bands (20 cm wide) 50 cm apart. Each band was covered with a clear polythene sheet coated with a viscous gum (Tanglefoot Co. Ltd., USA) to entrap small insects. The orientation of the sheets was marked and, from soil level, were designated 1 to 5. The sticky polythene sheets were removed weekly, and trapped whiteflies were counted and recorded.

3.3 RESULTS OF VIRUS STUDIES

3.3.1 Surveys, Identification and Characterisation of Kenyan Viruses

3.3.1.1 Visual Surveys for Sweet Potato Virus Disease Symptoms in Kenya and Central Uganda

All the important sweet potato growing regions in Kenya were surveyed, the locations of which are shown in Fig. 9. The results of the surveys are summarised in Table 9 which also includes further details of the locations in Kenya and Uganda, of the number of plots and their average sizes at each location, the origin and age of the stock, local names and colours of cultivars, predominant virus symptoms observed in the fields, estimated field incidence and farmers comments on the performance of their crops. An appraisal of these results indicates that sweet potato is generally grown as a subsistence crop on small plots ranging from 1/8 ha in high potential areas of the Central Province to 1 ha in Western Kenya where it is both an important food and cash crop. In Western and Nyanza Provinces of Kenya and in Central Uganda, sweet potato is an important stable food crop. Overall, in only two areas surveyed (Kambondo, in South Nyanza and Shiba Hills in Coast Province) were plots larger than 1 ha, and used to grow sweet potato as the only cash crop. The planting materials were originally obtained from neighbouring farmers, and most growers had propagated the same stocks for 10 to 20 years. However, due to population pressure, some people have migrated during the last 5 years from diverse locations to rather dry areas such as Rumuruti and Nanyuki in the Rift Valley Province, and introduced sweet potatoes there. The cultivar Kalamu Nyerere, a recent introduction from Tanzania, is currently the most popular commercial cultivar in the Kambondo area of South Nyanza. Many apparently similar cultivars had different names in different locations. The few cultivars grown at each location usually

included one producing red skinned and one producing white skinned tubers. Most farmers had originally grown more cultivars, but some of these were no longer cultivated due to degeneration, weevils and unsuitability. In Central Province and many other regions, the predominant cultivar Gorobu had red skinned tubers; this is a late maturing variety that is ideal for perennial cropping. The white skinned cv. Gikanda was also widely grown in Central Province. In Western Province, Coast Province and some parts of Eastern Province where sweet potatoes are grown as annual crops due to weevils or insufficient rainfall, short season white and red-skinned cultivars such as Toilo, Mania, and Mwezi-tatu are grown.

The predominant virus symptoms in field-grown sweet potatoes included chlorotic vein-banding, chlorotic spots, mottling and stunting; plants in the fields surveyed each had a range of symptoms, observations suggested the occurrence of mixed infections. The observations were supported by the results of virus identification tests. The estimated incidence of field infection was very interesting. Some areas, which included Kisii, Coast Province, and the NARC-Katumani station of KARI had very low levels (5-10%) of apparent infection. Two other areas, which included the new schemes in the dry areas of Nanyuki, Rumuruti and Maseno, also had low apparent infection levels (10-20%). In most other areas, in which 20-40% obvious infection was common, individual farmers "positively select" (i.e. for vigour/good health etc.) propagation stocks. Some areas with very high infection levels (60-100%) included scientific institutions of KARI (RRC-Kakamega, RRC-Embu, NARC-Kitale) in Kenya, Namulonge Research Station, and Makerere University (data for Makerere University not included) in Uganda, in which selected propagation stocks had not been used.

All farmers interviewed emphasised the need to increase sweet potato production and clearly indicated the problems limiting production. Although the problems differed in different locations, they generally included the lack of land for expansion in high potential areas, lack of locally adapted cultivars for dry areas and new schemes, lack of pest resistant cultivars, especially in areas where weevils are serious pests, and marketing difficulties.

Table 9. VISUAL SURVEYS FOR SWEET POTATO VIRUS DISEASES SYMPTOMS IN KENYA AND UGANDA

Location no. of plots and average size	Origin and age of stocks	Name and tuber skin colour of the main local cultivars	Predominant field virus symptoms	Estimated field infection (%)	Farmers main comments on crop performance
Coast Prov. 10-plots, Mombasa-1/4 ha Malindi (Shiba Hills) -1 ha	Mainly local; 10-12 yr	Mpeketoni, Taveta, Mukizoni, Ex. Kaloleni (red and white skins)	Yellow vein banding and chlorotic spots	8-10	Good market in Mombasa, * serious weevil problems. Early cultivars (4 months or less) preferred
Kiboko 2 plots 1 ha	Introduced from Machakos; c. 5 yr	names unknown (red and white skins)	stunting, limited vein banding chlorotic leaves	20-30	High potential of increasing production, but problems of rapid degeneration and lack of planting stocks
Machakos 4-plots 1/4 ha	Local; c. 10-20 yr	Mwezi-tatu others names unknown (red and white skins)	Vein banding mottling, stunting and chlorotic leaves	NARC-KARI 5-10 other areas 20-30	as above

Table 9. (continued)

Location no. of plots and average size	Origin and age of stocks	Name and tuber skin colour of the main local cultivars	Predominant field virus symptoms	Estimated field infection (%)	Farmers main comments on crop performance
Embu 3-plots 1/2 ha	Introduced from Meru; 10-20 yr	Muibai, Musinya, Bisale and Maragua (red and white skins)	Mottling, vein banding, stunting and chlorotic spots	30-40	High potential for increase but problem of marketing and lack of suitable cultivars
Meru 6-plots 1/2 ha	Local, more than 20 yr	Musinya, Gorobu Gikanda	Vein banding stunting, chlorotic leaves and star-like spots	20-30	High production and consumption potential to be a commercialised commodity crop
Nyeri 15-plots 1/8 ha	Mainly local but some new introduction from western Kenya; 10-20 yr	Gorobu, Gikanda local red and white skins respectively. Mwezi-tatu, new introduction in less than 10 yr	Vein banding stunting, chlorotic spots	High grounds 10-20 Lower ground 20-30	Great potential to increase for food and animal feeds; high population pressure and less land availability favour increase as an alternative to maize
Kirinyaga 12-plots 1/4 ha	Local but also from Embu and Meru; over 10 yr	Gorobu-red skin Mwezi-tatu -white Gikanda-white	Vein banding, mottling, stunting, chlorosis ring-spots	30-40	Limited number of suitable cultivars; potential to increase production but need for market, also rapid cultivars degeneration

Table 9. (continued)

Location no. of plots and average size	Origin and age of stocks	Name and tuber skin colour of the main local cultivars	Predominant field virus symptoms	Estimated field infection (%)	Farmers main comments on crop performance
Kiambu 16-plots 1/8 ha	Local; over 10 yr	Gorobu, Musinya Gikanda	Vein banding, mottling, chlorotic leaves stunting round star-like spots	30-40	High potential to increase production mainly for animal feed but limited land available. Need for market expansion. Problem of degeneration and lack of suitable cultivars
Muguga 4-plots 1/8 ha	Local; over 10-yr	Gorobu, Gikanda white and red skins	Vein banding chlorotic spots and stunting	30-40	as above
Nanyuki 6-plots 1/4 ha	Introduction from Meru, Nyeri, and Machakos; 5-10 yr	Gikanda, Gorobu red and white skins	mild mottling and vein bandling	10-20	Marginal areas; farmers wish to increase production and area, but lack of planting stocks of suitable cultivars
Rumuruti 6-plots 1/4 ha	Introduction from neighbouring regions Nyeri and Nanyuki; 5-10 yr	Gilkanda, Muita, Gorobu, Muburi	stunting, mottling and chlorotic spots	10-20	as above

Table 9. (continued)

Location no. of plots and average size	Origin and age of stocks	Name and tuber skin colour of the main local cultivars	Predominant field virus symptoms	Estimated field infection (%)	Farmers main comments on crop performance
NARC-KARI Kitale Collection 8 adjacent plots 1/8 ha	Introduction from Maseno and Kakamega; 5-10 yr	Toilo, Muibai, Bisale Nyalusyo, Namale Nyaheki Maseno	Very serious stunting and chlorosis, vein bandling, mottling and chlorotic starlike spots	Toilo * 5-10 Maseno-14 30-40% others 80-100	Very serious germplasm degeneration and need for virus tolerant cultivars. Need to increase production for alternative food and animal feed, but lack of planting stocks and suitable cultivars
Maseno 5-plots 1/2 ha	Local and some introduced from Kakamega and Uganda; 10-20 yr	Nylon, Mosila, Emiekhanyu Musinyama from Uganda. Red and white skins	Vein bandling, mottling, stunting and chlorotic spots	10-20	Need for greater market and better yielding cultivars
South Nyanza (Kambondo) 4-plots 1 ha	Local and introduced from Tanzania; 10-20 yr	Kalamu-Nyerere commercial cultivar from Tanzania red skin. Others local, Tomla Rotha-white	Stunting, vein bandling, chlorotic leaves and chlorotic spots	40-50	Good market, concern over the rapid rate of degeneration of the commercial cultivar

Table 9. (continued)

Location no. of plots and average size	Origin and age of stocks	Name and tuber skin colour of the main local cultivars	Predominant field virus symptoms	Estimated field infection (%)	Farmers main comments on crop performance
Kisii 4-plots 1/2 ha	Local and introduced from Kakamega	Nyarkomolo, Nyakonde Nybukande Nyarkoguta and Nyamungu White and red skins	Frequent symptom masking due to prolific growth; moderate vein clearing, mottling and chlorotic spots	5-10	Very high potential area for sweet potato but marketing problems. Need for better performing cultivars
Kakamega 4-plots 4 ha Women co-operative farms of 3x3 m plot per woman. RRC-KARI 2-plots, 1/2 ha	Local and introductions; 10-20 yr	Toilo, Nyalieki, Mania, Nyaluoya, Bisale, Mafuta, Mwezi-tatu, Musinya White red skins	Vein bandling, mottling stunting, chlorotic leaves some chlorotic ring-spots	Farmers fields 30-40 RRC-KARI 60-80	Local cultivars very highly tolerant to degeneration and weevil damages, but having relatively low yields. Good market but need for better cultivars
Busia 7-plots 1/4 ha	Local but introduction from Kakamega and Uganda; 10-20 yr	Local names unknown except Msinya from Kakamega Red and white skins	High inteveinal chlorotic ring-spots stunting and chlorosis	30-40	Sweet potato and cassava are stable foods here; degeneration and low yields are problems. Need for better cultivars

Table 9. (continued)

Location no. of plots and average size	Origin and age of stocks	Name and tuber skin colour of the main local cultivars	Predominant field virus symptoms	Estimated field infection (%)	Farmers main comments on crop performance
Uganda Namulonge Research Station 2-plots 1/2 ha	Local cultivars; over 20 yr. Introduction from Tanzania; 10-20 yr	Kawogo, Wagabolige Nantongo Bitamisi Sukali Kyeibandulei Red and white skins	Vein banding, mottling stunting chlorotic leaves.	70-80	Very serious cultivars degeneration; local cultivars tolerant, but some IITA-Nigeria introductions being lost due to degeneration and very low yields. Need for resistant cultivars
Namulonge 3-plots 1/4 ha	Local cultivars; 10-20 yr	Sukali, Kyeibandulei some unknown names	as above	30-40	Serious degeneration and loss of cultivars. Need for better yielding suitable cultivars
Mukono 2-plots 1/4 ha	Local cultivars; over 20 yr	Kaongo other unknown names Red and white skins	as above	20-30	Low yields due to lack of suitable cultivars and degeneration

Fig. 9

AREAS OF KENYA SURVEYED FOR SWEET POTATO VIRUS DISEASES, 1989 - 1990

3.3.1.2 Viruses detected in Sweet Potato Samples Collected During Surveys

The frequency (%) of the seven viruses in locations surveyed in Kenya and Uganda are shown in Table 10. Generally, there was little variation in the levels of infection of a particular virus within locations of one Province except where major geographic or climatic differences existed. However, SPRSV was not detected in Coast Province, Eastern Province, Rift-Valley Province, Central Uganda, in two locations of Western/Nyanza Provinces and Central Province. Similarly, SPC-LV was not detected in several locations (Table 10). Kirinyaga and Kiambu in Central Province, Busia, Kisii and Kakamega in Western Province were areas in which all the seven viruses were detected and which had very high virus incidences (Table 10). The occurrence of the seven viruses in the major Provinces is summarised in Table 11. These results show that Coast Province was the region of lowest infection in Kenya, although SPLV (60%) occurred commonly, but symptomlessly, in most cultivars; visual field surveys and estimation of field infection had earlier indicated that this was the Province of lowest (8-10%) infection. In the Eastern Province of Kenya, which includes marginal areas (Kiboko and Machakos) and medium and high potential areas (Embu and Meru, respectively), levels of infection were moderately high with either some viruses not occurring or important viruses (SPFMV and SPMMV) occurring relatively rarely. Levels of infection in the Rift-Valley Province were also moderately high in contrast to the adjacent Central and Western/Nyanza Provinces. Central Uganda, however, was the area of highest virus incidence.

The common occurrence in sweet potatoes of virus complexes was indicated during field surveys by the diverse symptoms in individual plants, and confirmed by virus indentification tests. The occurrence of complexes in all locations surveyed is shown in details in Table 12, and summarised for the six Provinces in Table 13. Figures 10 to 15 also show graphically the common occurrence of the virus

complexes in the Kenyan Provinces. The minimum complex of two viruses was common in the Coast and Eastern Provinces, regions of relatively low infection; in both, SPLV was the commonest component of complexes, and both had high numbers of samples (26 and 41% respectively) containing no detectable viruses. In these two areas, two viruses occurred in 11-15% of samples, three viruses in 5% and four in 1%. Virus complexes occurred in relatively few samples (all 24% contained two viruses) from the Rift-Valley Province. The highest incidence of complexes of two viruses was highest in Western/Nyanza Provinces (31%) followed by Rift-Valley (24%), while in other Provinces of Kenya between 10-15% of samples contained two viruses. Although samples from Central Uganda had the lowest incidence (10%) of dual infection, they had the highest incidence of complexes of 4 and 5 viruses.

The occurrence of complexes of three viruses was highest in Western/Nyanza Provinces (15%) followed by the Central Province (13%), Central Uganda (11%) and the other Provinces (5-9%).

Central Uganda had the highest occurrence of complexes of four viruses (25%) followed by Central Province of Kenya (12%); the incidence of similar complexes in other Provinces was between 1-7%. Central Uganda also had the highest incidence of complexes containing five viruses (22%), followed by Central Province of Kenya (16%), while Eastern and Western/Nyanza Provinces each had similar complexes in only 2% of samples; such complexes were not detected in the Coast and Rift-Valley Provinces. Complexes of six viruses were detected only in Central Uganda (11%), Central Province of Kenya (8%) and Western/Nyanza Provinces (2%). Complexes of seven viruses were detected in Central Province of Kenya (6%) and in Western/Nyanza Provinces (3%).

CMV was found to occur only in combination with SPFMV and/or SPLV in all locations surveyed (Table 14). The incidence of the complexes was dependent on

the prevalence of SPFMV and/or SPLV. Where relatively similar numbers of both viruses occurred, as in Central Uganda, their frequency with CMV was similar (Table 14). Where the incidence of SPLV was greater, as in Coast Province, combination with CMV were more frequent with SPLV (Table 14) than with SPFMV, or both. However, when SPFMV occurred more prevalently that SPLV, CMV tended to combine most frequently with both viruses, less frequently with SPFMV, and least with SPLV (e.g. in Kirinyaga, Kiambu, and KARI Muguga locations, Table 14). In dry areas (Machakos, Rumuruti and Nanyuki), which apparently also had higher incidences of SPLV, CMV commonly occurred in complex with SPLV.

In the Coast, Eastern, and Rift-Valley Provinces, CMV occurred mainly in complex with SPLV, and in Central, Western/Nyanza Provinces and Central Uganda with both viruses (Table 15). Overall, CMV was found in combination with SPFMV in 15% of samples, with SPLV in 50%, and with both viruses in 35%.

Table 10 RELATIVE OCCURRENCE OF SEVEN VIRUSES IN AREAS SURVEYED IN KENYA AND UGANDA

	Samples (%) containing							
Location	Samples (No.)	SPFMV	SPLV	SPMMV	SPC-LV	SPRSV	CMV*	SPCSV
COAST PROV.								
Mombasa/ Malindi	120	6	60	2	0	0	15	8
EASTERN PROV.								
Kiboko	33	6	33	6	0	0	32	42
Machakos	46	28	99	20	11	0	33	27
Embu	38	8	26	8	8	0	21	25
Meru	33	33	58	6	18	0	24	39
CENTRAL PROV.								
Nyeri	63	11	56	6	1	0	23	11
Kirinyaga	60	97	78	72	53	33	55	67
Kiambu	60	88	60	65	23	5	62	77
KARI-Muguga	60	80	62	22	0	0	55	20
RIFT-VALLEY PROV.								
Nanyuki	45	22	64	42	2	0	7	7
Rumuruti	36	3	64	17	0	0	25	11
Kitale	150	36	55	21	17	0	80	90
WESTERN / NYANZA PROV.								
Maseno	120	71	52	13	0	0	34	21
S/Nyanza	95	39	74	6	0	0	62	72
Kisii	60	77	85	37	28	17	53	57
Kakamega	107	59	78	29	6	8	53	67
Busia	60	7	33	7	40	20	48	57
UGANDA								
Namulonge St.	58	71	53	62	33	0	50	71
Namulonge Loc.	49	67	65	53	16	0	67	78
Mukono Loc.	54	87	57	59	39	0	39	67
Total	1377	896	1212	553	295	83	838	914
Mean	69	45	61	28	15	4	42	46

N.B.: Percentages greater than 100 for some locations is due to occurrences of virus mixtures.
* CMV found only in complex with SPLV and/or SPFMV.

Table 11 RELATIVE OCCURRENCE OF SEVEN VIRUSES OF SWEET POTATOES DETECTED IN FIELD SURVEYS IN KENYA AND UGANDA

Provinces	Samples No.	Occurrence (%) of each virus *						
		SPFMV	SPLV	SPMMV	SPC-LV	SPRSV	CMV**	SPCSV
Coast	120	6	60	2	0	0	15	8
Eastern	150	19	54	10	9	0	28	33
Central	273	69	64	41	19	10	49	44
Rift Valley	231	20	61	27	6	0	37	36
Western/Nyanza	442	51	64	18	15	9	50	55
Central Uganda	161	75	58	58	29	0	52	52
Mean	-	40	60	26	13	3	39	41

* Viruses frequently occurred in complexes.

** CMV found only in complex with SPLV and/or SPFMV.

Table 12 SAMPLES (%) WITH MULTIPLE VIRUS INFECTIONS IN AREAS SURVEYED

Province	Location	Samples (No.)	No. of viruses per sample (%)						
			1	2	3	4	5	6	7
COAST	Mombasa/Malindi	120	50	17	5	1	0	0	0
EASTERN	Kiboko	33	27	6	3	0	0	0	0
	Machakos	46	73	9	0	0	0	0	0
	Embu	38	18	13	0	0	0	0	0
	Meru	33	36	15	15	9	6	0	0
CENTRAL	Nyeri	63	46	19	2	0	0	0	0
	Kirinyaga	60	12	8	15	8	13	18	25
	Kiambu	60	10	5	8	18	35	15	0
	KARI-Muguga	60	8	25	27	22	15	0	0
RIFT-VALLEY	Nanyuki	45	49	31	2	7	0	0	0
	Rumuruti	36	50	17	11	0	0	0	0
	Kitale	150	35	23	15	7	0	0	0
WESTERN/NYANZA	Maseno	120	31	28	21	6	4	0	0
	S/Nyanza	95	31	51	4	1	0	0	0
	Kisii	60	17	12	17	15	8	10	13
	Kakamega	107	17	38	14	6	0	0	0
	Busia	60	20	25	18	0	0	0	0
UGANDA	Namulonge	58	19	17	9	16	26	10	0
	Namulonge	49	8	10	16	29	27	4	0
	Mukono	53	15	2	8	30	13	20	0
Total		1343	572	371	199	175	147	77	38
Mean		67	27	19	10	9	7	4	2

Table 13 INFECTION OF SWEET POTATOES WITH VIRUS COMPLEXES IN KENYA AND UGANDA

Province	Samples	Viruses per sample (%) *							
	(No.)	0	1	2	3	4	5	6	7
Coast	120	26	50	15	5	1	0	0	0
Eastern	150	41	39	11	5	2	2	0	0
Central	273	10	19	14	13	12	16	8	6
Rift Valley	231	18	47	24	9	5	0	0	0
Western/Nyanza	442	17	23	31	15	7	2	2	3
Central Uganda	161	7	14	10	11	25	22	11	0
Mean	-	20	32	18	10	9	7	4	2

* The viruses tested for were: SPFMV, SPMMV, SPC-LV, SPRSV, SPCSV, SPLV, and CMV.

Table 14 FREQUENCY OF CMV IN RELATION TO THAT OF SPFMV AND SPLV

Province	Location	Samples (No.)	No. with CMV and			Total with CMV
			SPFMV	SPLV	SPFMV +SPLV	
COAST	Mombasa/Malindi	120	1	16	1	18
EASTERN	Kiboko	30	0	11	0	11
	Machakos	46	0	15	0	15
	Embu	38	2	6	2	8
	Meru	33	3	2	3	8
CENTRAL	Nyeri	63	1	17	3	21
	Kirinyaga	60	5	0	28	33
	Kiambu	60	12	0	25	37
	KARI-Muguga	60	6	5	22	33
RIFT-VALLEY	Nanyuki	45	1	2	0	3
	Rumuruti	36	0	9	0	9
	Kitale	150	28	52	12	92
WESTERN/NYANZA	Maseno	120	8	11	22	41
	S/Nyanza	95	9	35	13	57
	Kisii	60	1	0	31	32
	Kakamega	107	2	10	45	57
	Busia	60	0	29	0	29
UGANDA	Namulonge	58	5	6	18	29
	Namulonge	49	4	9	20	33
	Mukono	54	5	2	18	25

Table 15 FREQUENCY OF CMV IN RELATION TO THAT OF SPFMV AND SPLV SUMMARISED BY PROVINCE.

Province	Samples (No.)	No. with CMV* and			Total with CMV
		SPFMV	SPLV	SPFMV +SPLV	
Coast	120	1 (6)	16 (88)	1 (6)	18
Eastern	150	5 (12)	34 (76)	5 (12)	42
Central	273	24 (19)	22 (18)	78 (63)	124
Rift Valley	231	29 (28)	63 (60)	12 (12)	104
Western/Nyanza	442	20 (10)	85 (39)	111 (51)	216
Central Uganda	161	13 (15)	17 (20)	56 (65)	87
Mean (%)	-	15	50	35	100

* Numbers in parentheses are percentages.

Fig. 10. SURVEYS SHOWING THE COMMON OCCURRENCE
IN SWEET POTATOES OF VIRUS COMPLEXES
COAST PROVINCE

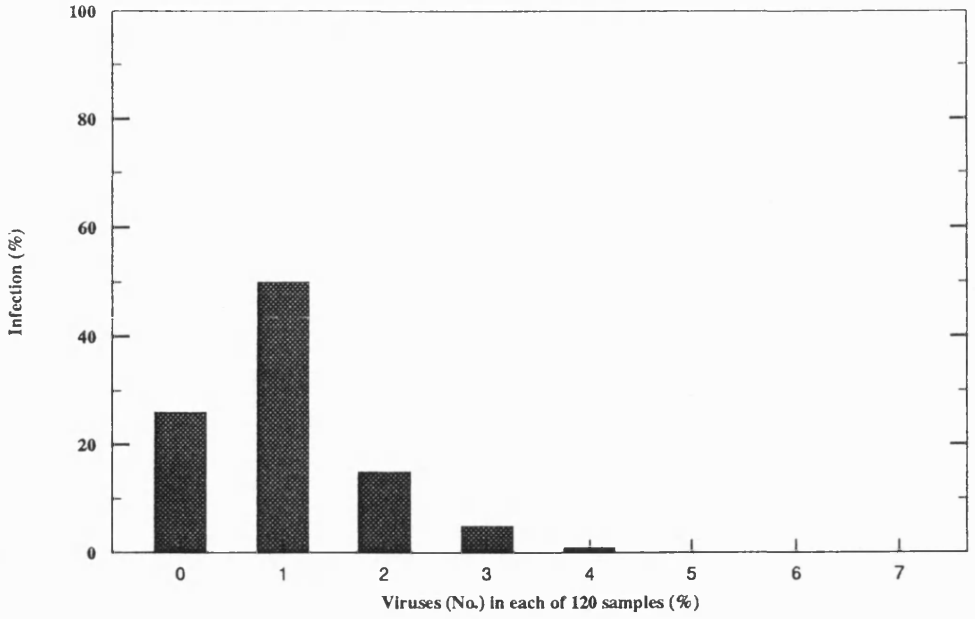


Fig. 11. SURVEYS SHOWING THE COMMON OCCURRENCE
IN SWEET POTATOES OF VIRUS COMPLEXES
EASTERN PROVINCE

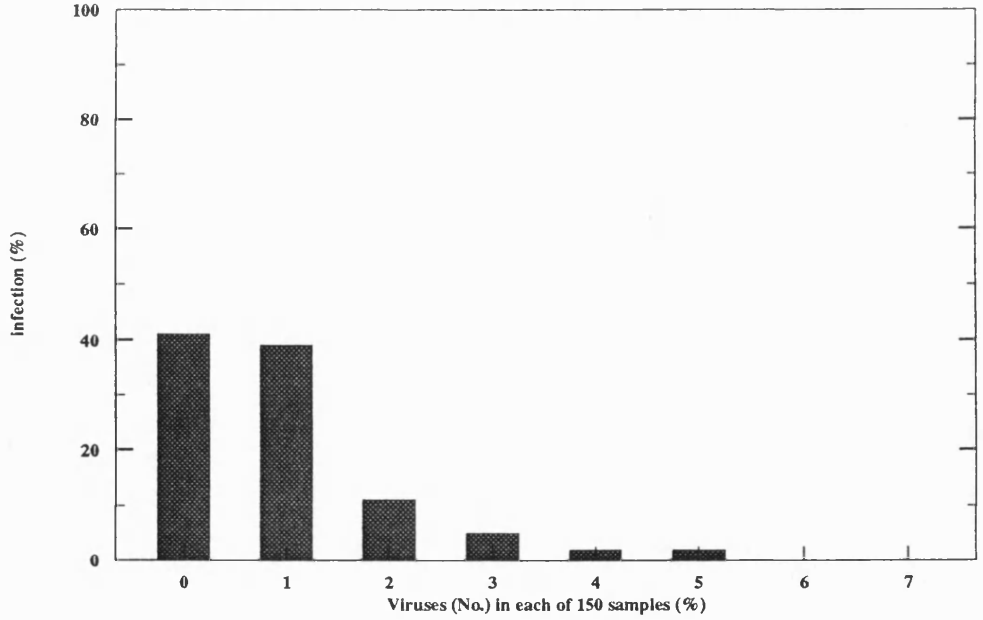


Fig. 12. SURVEYS SHOWING THE COMMON OCCURRENCE IN SWEET POTATOES OF VIRUS COMPLEXES CENTRAL PROVINCE

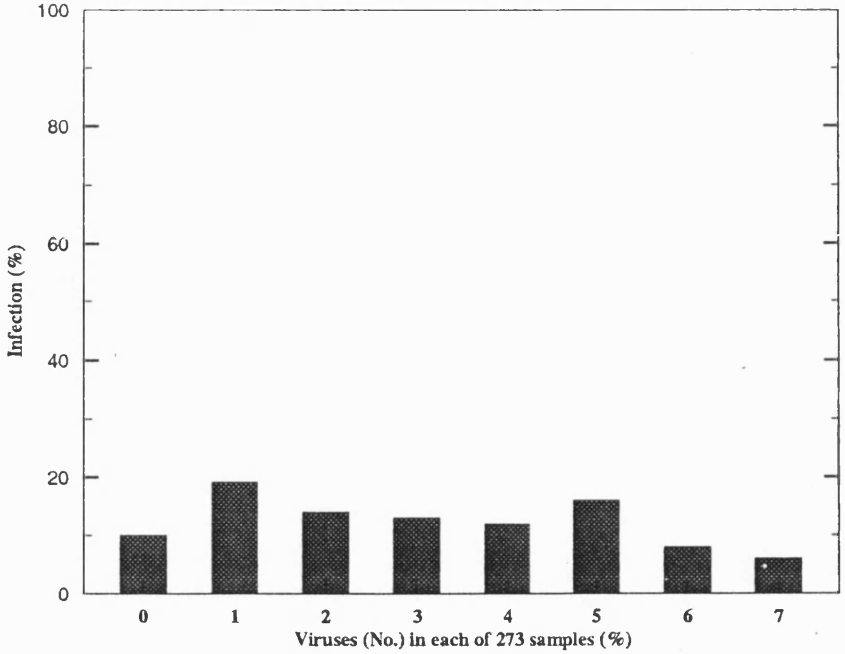


Fig. 13. SURVEYS SHOWING THE COMMON OCCURRENCE IN SWEET POTATOES OF VIRUS COMPLEXES RIFT VALLEY PROVINCE

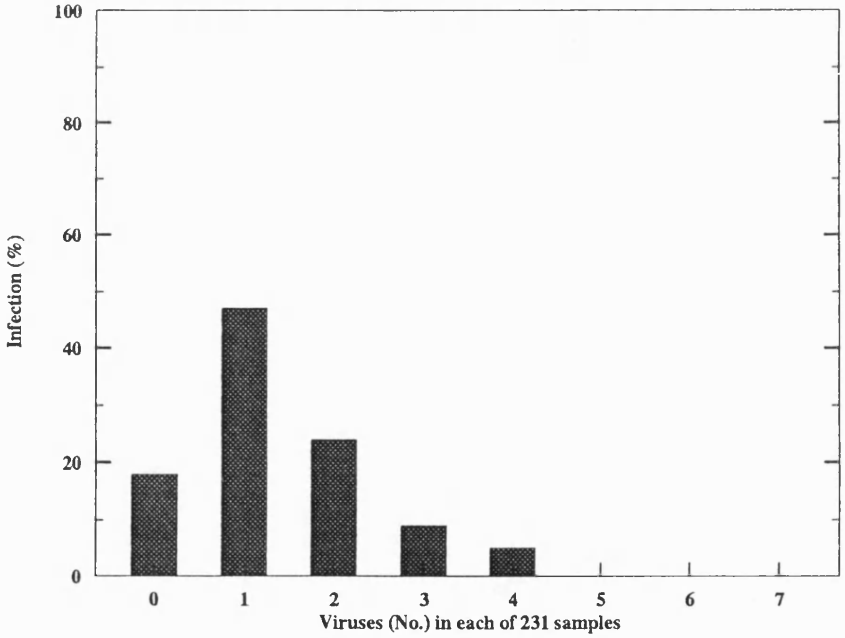


Fig. 14. SURVEYS SHOWING THE COMMON OCCURRENCE IN SWEET POTATOES OF VIRUS COMPLEXES WESTERN/NYANZA PROVINCES

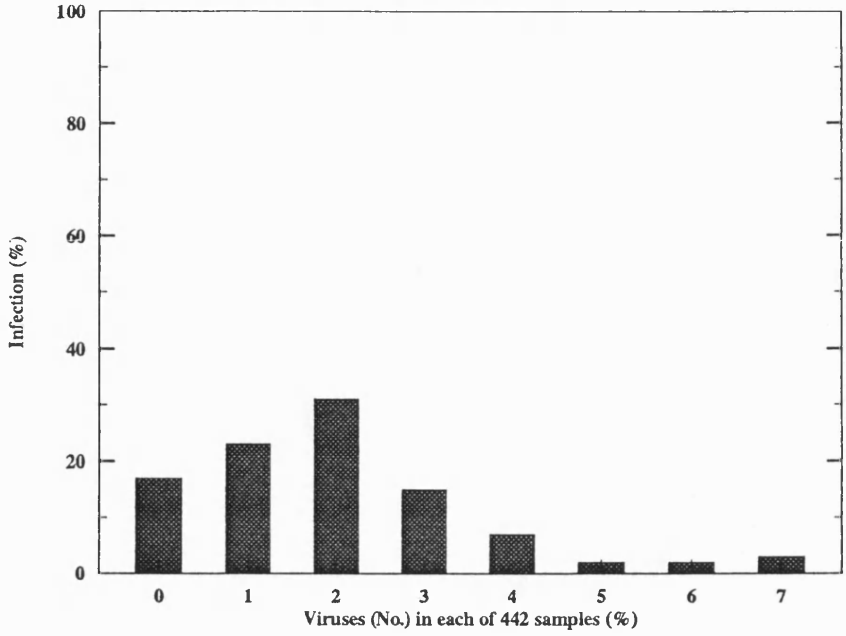
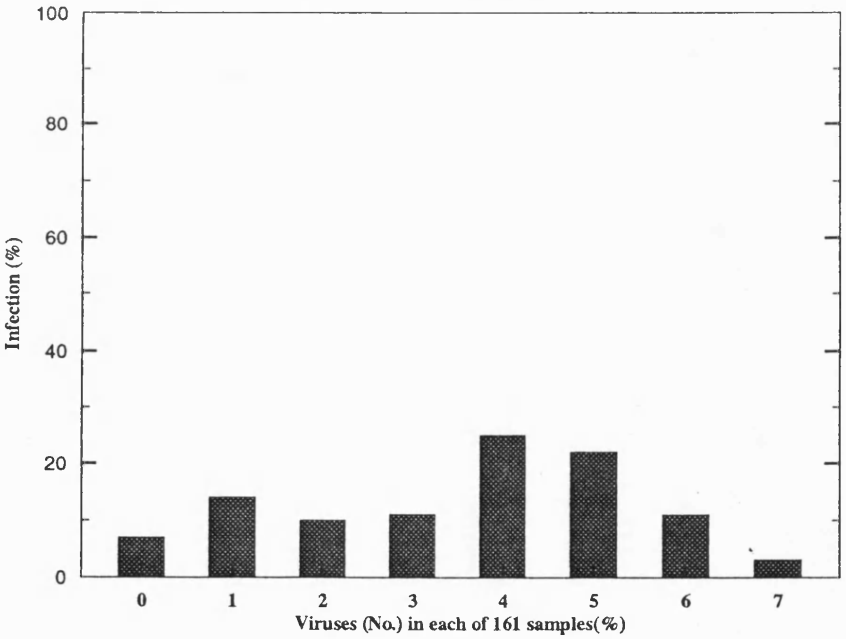


Fig. 15. SURVEYS SHOWING THE COMMON OCCURRENCE IN SWEET POTATOES OF VIRUS COMPLEXES CENTRAL UGANDA



3.3.1.3 Partial Characterisation of Viruses

Except for a single isolate of SPMMV (Hollings *et al.*, 1976a & b), the viruses infecting sweet potatoes in Kenya have not previously been investigated adequately and compared with isolates occurring elsewhere. Attempts reported here were made therefore, to further characterise some of the viruses.

3.3.1.3.1 Sweet potato caulimo-like virus

Isolation and Transmission Tests:

During the field surveys (3.3.1.2), SPC-LV was commonly found in sweet potatoes throughout Kenya (Table 10). Although it often occurred in complexes with other viruses, it was found to occur alone in some plants at Embu. These plants, therefore, served as initial sources of virus for subsequent investigations.

Scions from an infected sweet potato were grafted to healthy *I. setosa* seedlings. After 4-5 wk, the immature leaves of 5 of 12 of the graft-inoculated seedlings developed interveinal chlorotic spotting and scattered vein-banding (Fig. 16Ia). These symptoms later intensified and often the leaves soon after became completely chlorotic; soon after, the seriously affected leaves died (Fig. 16b & c), presumably due to the occlusion of xylem vessels caused by virus-induced invaginations of xylem parenchyma cells as described by Atkey and Brunt (1987).

Attempts to transmit the virus mechanically from infected to healthy *I. setosa* seedlings using a wide range of extractants failed; the virus also failed to infect mechanically inoculated seedlings (3-5 of each species) of *I. nil, Chenopodium amaranticolor, C. quinoa, Capsicum annuum, Nicotiana benthamiana, N. clevelandii, N. glutinosa, N. tabacum, Phaseolus vulgaris, Pisum sativum, Vicia faba and Vigna unguiculata.*

The virus was not transmitted in the non-persistent or semi-persistent manner

from infected to healthy the *I. setosa* by either of two aphid species (*Aphis gossypii and Myzus persicae*). Using 25-30 aphids per test plant, *A. gossypii* failed to transmit the virus in the non-persistent manner to any of 11 plants and *M. persicae* to any of 9 plants. Both species each failed to transmit the virus in the semi-persistent manner to any of 8 test plants.

Identity of Virus:

The virus, extracted and purified from infected *I. setosa* leaves by two cycles of differential centrifugation, contained isometric particles mostly c. 50 nm in diameter (Fig. 16-IIa). No virus particles were found by electron microscopy in negatively-stained sap from infected *I. setosa* leaves. However, isometric particles c. 50 nm in diameter were readily detected in similar leaf extracts which were mounted on electron microscope grids coated with antiserum to SPC-LV. "Trapped" particles were also seen to be coated with antibody when decorated with antiserum to SPC-LV (Fig. 16-IIb).

In further comparative tests, the number of particles present in *I. setosa* sap mounted on grids coated with pre-immune rabbit serum (the controls) and those coated with homologous antiserum were recorded in three assays, in each of which the average number of particles were calculated from seven to ten counts in different grid areas. Some grids on which particles were trapped were subsequently exposed to antiserum to cauliflower mosaic (CIMV), dahlia mosaic (DMV), carnation etched ring (CERV) or soybean chlorotic mottle (SoyCMV) viruses and, as controls, pre-immune serum.

The particle counts showed that grids coated with antiserum to SPC-LV trapped c. 30-fold more particles than those coated with pre-immune serum (Table 16). In the "decoration" tests, "trapped" particles were heavily "decorated" with antibodies to SPC-LV (Fig. 16-IIb) but not with those to any of the other four caulimoviruses.

Although these results provided good evidence that this isometric virus in Kenya was serologically very closely related to SPC-LV, this was confirmed by double gel diffusion tests. When purified virus from *I. setosa* was reacted in agar gels with antisera to SPC-LV, CIMV, DMV or SoyCMV, line of precipitate were obtained only in reaction between the virus and antiserum to SPC-LV.

Table 16. SPC-LV IMMUNOSORBENT ELECTRON MICROSCOPY

	Particles/grid square in assay			
	1	2	3	Overall mean
Control	0.33 *	0.38	0.50	0.40
Trapped	11.83	11.00	12.30	11.70
Trapped & decorated	12.63	8.39	18.00	13.00

* No. of particles per serum (mean of 7-10 counts) at a nominal magnification of x100,000.

3.3.1.3.2 Sweet potato mild mottle virus (SPMMV)

Isolation: SPMMV was shown to occur commonly throughout Kenya, often in severely affected plants also containing other viruses (3.3.1.2). The virus was readily isolated from naturally infected sweet potatoes (Figs. 17a,b) or, more easily from graft inoculated *I. setosa* plants (Fig. 17c) by mechanical inoculation of sap to its experimental hosts, especially *Nicotiana benthamiana, N. clevelandii, N. glutinosa* and/or *N. tabacum.*

All of the many SPMMV isolates obtained in routine isolation tests induced similar symptoms in indicator plants. One isolate from Kakamega, shown by ISEM

tests to be uncontaminated by other sweet potato viruses, was selected for more detailed investigations and was thereafter routinely propagated in *N. glutinosa* and *N. tabacum.*

Hosts and Symptomatology: When infected by mechanical inoculation *Nicotiana clevelandii* (Fig. 17e), *N. glutinosa* and *N. tabacum* (Fig. 17d) developed a few grey chlorotic and/or necrotic lesions in inoculated leaves and conspicuous chlorosis in systemically infected leaves; infected plants of *N. clevelandii* were also severely stunted (Fig. 17f). By contrast, inoculated plants of *Nicotiana benthamiana* and *N. megalosiphon* developed only a mild chlorosis of systemically-infected leaves.

Systemically-infected leaves of *Ipomoea nil, I. purpurea* and *I. setosa* (Fig. 17c) developed conspicuous chlorotic veinbanding and chlorotic mottling after c. 2 weeks, but leaves produced subsequently were often infected symptonlessly. Identical symptoms were induced in graft-inoculated *I. setosa* seedlings.

In inoculated *Chenopodium amaraticolor, C. quinoa, Gomphrena globosa* and *Tetragonia expansa* seedlings, SPMMV induced chlorotic local lesions but no systemic infection.

The virus failed to infect plants of *Atropa belladonna, Brassica pekinensis, Capsicum annuum, Cucumis sativus, Glycine max, Phaseolus vulgaris, Vicia faba and Vigna unguiculata.*

Purified virus preparations (2.5.7) contained numerous filamentous particles mostly c. 800-900 nm long which sedimented at c. 150 S. The particles were readily detected in infective sap and purified preparations by ISEM (Fig. 17-IIa), ELISA and, as described earlier (3.3.1.2), by NCM-ELISA.

In ISEM, in which particles were "trapped" by coating electron microscope grids with antiserum (1/1,000) to SPMMV and then "decorated" with homologous

or heterologous antiserum (1/50), the particles were heavily decorated with antiserum to SPMMV but not with those to SPFMV, SPLV, SPCSV, bean yellow mosaic, clover yellow vein, pepper veinal mottle, tobacco etch, turnip mosaic or watermelon mosaic 2 viruses. These results indicate that SPMMV is serologically unrelated to aphid-borne potyviruses.

3.3.1.3.3 Sweet potato feathery mottle virus (SPFMV)

Infection of sweet potatoes in Kenya by SPFMV was widespread (Fig. 18 a,b,c) and prevalent. Although the virus usually occurred together with other viruses, pure cultures of the virus were readily established by graft-inoculating *I. setosa* seedlings from which SPFMV was then selectively acquired by aphids (*Aphis gossypii or Myzus persicae*) and transmitted to healthy *I. setosa* seedlings. One typical isolate so obtained from a sweet potato plant at Muguga was selected for further investigation.

In graft-inoculated *I. setosa plants*, and those infected with viruliferous aphids, SPFMV induced chlorotic spotting (Fig. 19Ia) and conspicuous chlorotic banding of main leaf veins after 2-3 weeks (Fig. 19Ib); leaves produced subsequently were usually infected symptomlessly. The virus was transmitted mechanically in three tests to a total of 11 of 24 *I. setosa* seedlings and to 15 of 22 *I. nil* plants. Young acutely infected *I. nil* plants (Fig. 19Ic) were found to be good sources of inocula; such inocula infected all of 24 *I. nil* seedlings and 16 of 22 *I. setosa* seedlings. The virus, however, failed to infect any mechanically inoculated seedlings (3-5 of each species) of *Nicotiana benthamiana, N. clevelandii, N. glutinosa, N. megalosiphon, N. tabacum Chenopodium amaranticolor, C. quinoa, Gomphrena globosa* or *Tetragonia expansa.*

The virus was graft-transmitted from *I. setosa* to four virus-free plants each of *I. batatas* cvs Rose Centennial and Brondal. After 10-12, weeks a few leaves of two

plants of each cultivars produced some chlorotic spots (Fig. 18d,e) which later developed maroon-coloured rims (Fig. 18f). Leaves produced subsequently were usually symptonless.

Purified preparations of the virus, obtained as described previously (2.5.7) contained numerous filamentous particles; these were often aggregated so could not be measured easily. However, particles in *I. nil* sap were mostly 750-775 nm long (Fig. 19IIa). In ISEM, the particles were heavily decorated with antiserum (1/50) to SPFMV received from Dr. J. Moyer and with those to SPFMV isolates from South Africa, New Zealand and Kenya available in the antiserum reference collection at the Horticulture Research International, Littlehampton. In similar tests, however, the SPFMV particles were not decorated by antiserum (1/50) to SPMMV, SPLV, SPCSV bean yellow mosaic, clover yellow vein, pepper veinal mottle, potato Y, turnip mosaic or watermelon mosaic 2 viruses.

3.3.1.3.4 A putative virus-like agent

During the third season of the surveys, *I. setosa* plants graft-inoculated with scions of severely diseased sweet potatoes (Fig. 20a) from Nyeri, initially developed chlorotic vein-banding leaf symptoms. However, during the following 3-5 weeks the test plants developed leaves with much reduced laminae and were very stunted (Figs 20b,c,d,e); these symptoms are very similar to those induced in *I. setosa* by SPFMV and the uncharacterised whitefly transmitted, non-mechanically transmissible virus-like agent in West Africa and Israel (Moyer and Salazar, 1989).

Only SPMMV could be detected in such plants by back-tests to indicator plants and by ISEM decoration tests with antisera to all viruses detected previously in Kenya sweet potatoes (3.3.1.2). Thus, symptoms typical of SPMMV were induced in mechanically-inoculated *N. glutinosa* and *N. tabacum*, and the identity of the virus

inocula from the severely affected *I. setosa* plants to healthy *I. setosa* resulted in the production of only chlorotic vein-banding systemic leaf symptoms. These results suggest that SPMMV can occur in complex with a virus-like agent in severely affected sweet potatoes in Kenya, a situation analogous to that in West Africa and Israel.

Fig. 16I. Symptoms induced by sweet potato caulimo-like virus (SPC-LV) in *Ipomoea setosa* after graft transmission from sweet potato.

a. Initial symptoms of general chlorosis after graft transmission.

b. Broad vein chlorosis.

c. Extensive leaf chlorosis leading to the death of the leaf.

Fig. 16-I

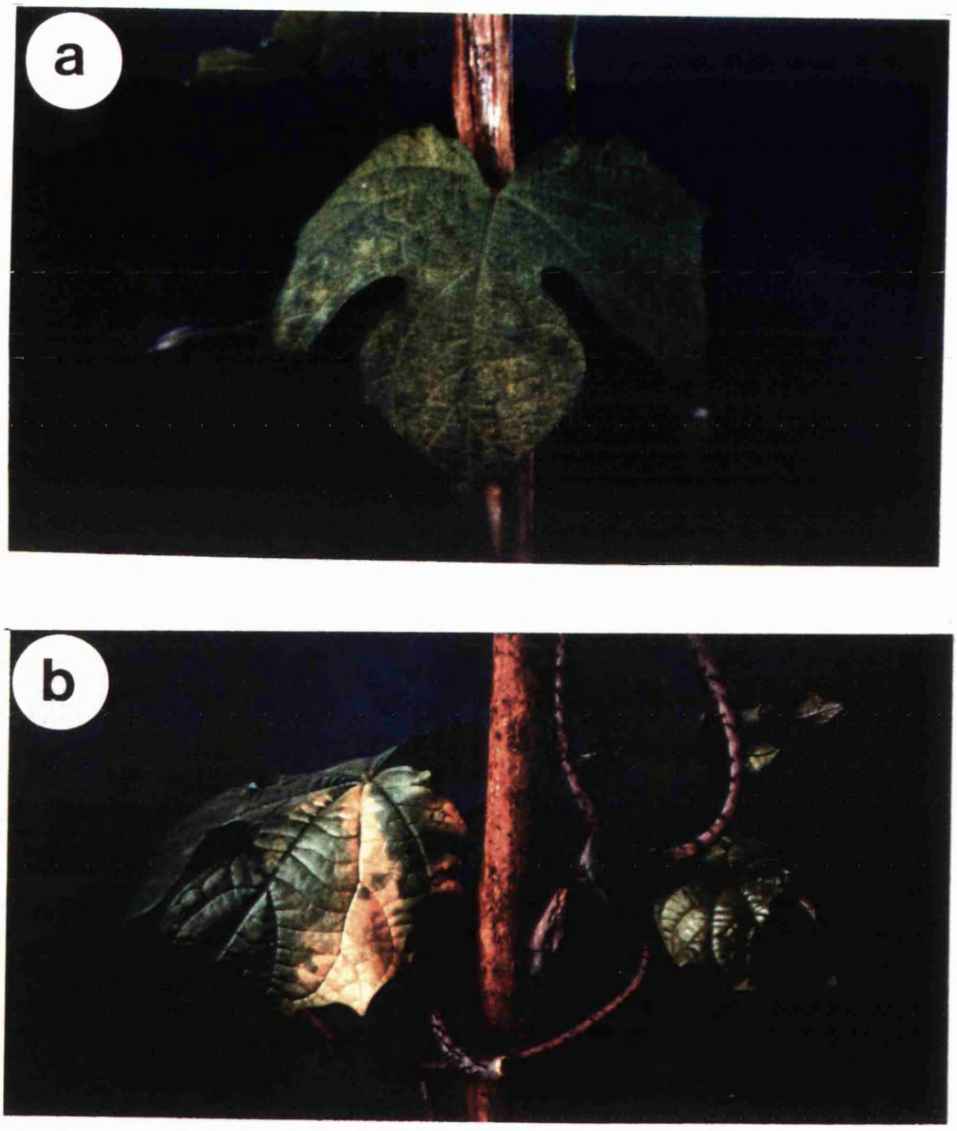
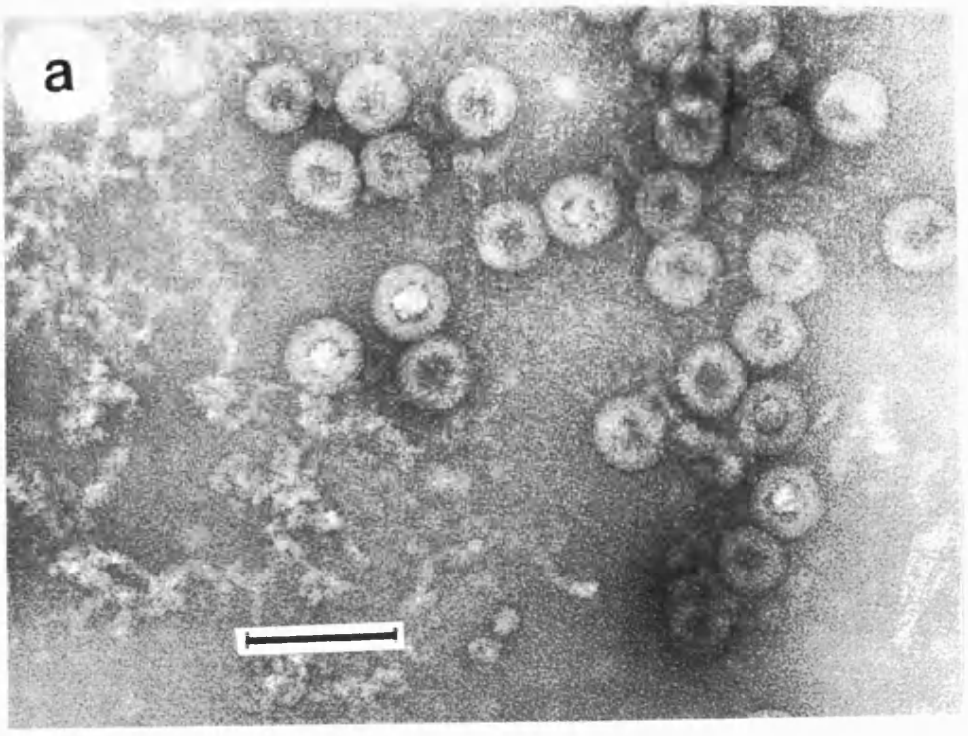
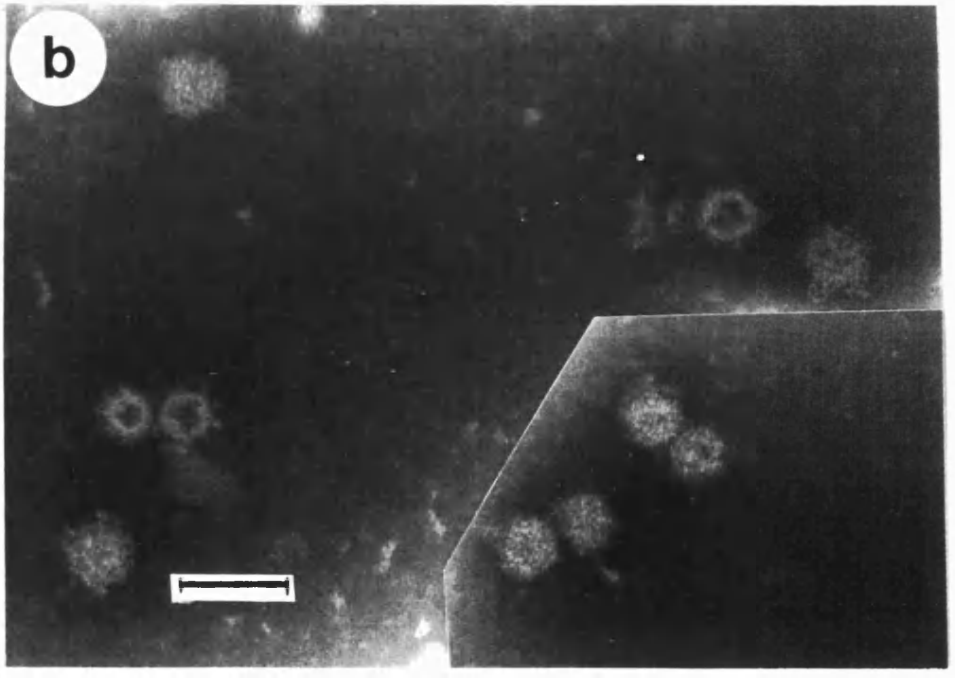


Fig-16-II 16II. Particle morphology of SPC-LV.



a. SPC-LV. Purified virus Bar scale represents 100 nm .



b. SPC-LV. Decorated particles Bar scale represents 100 nm .

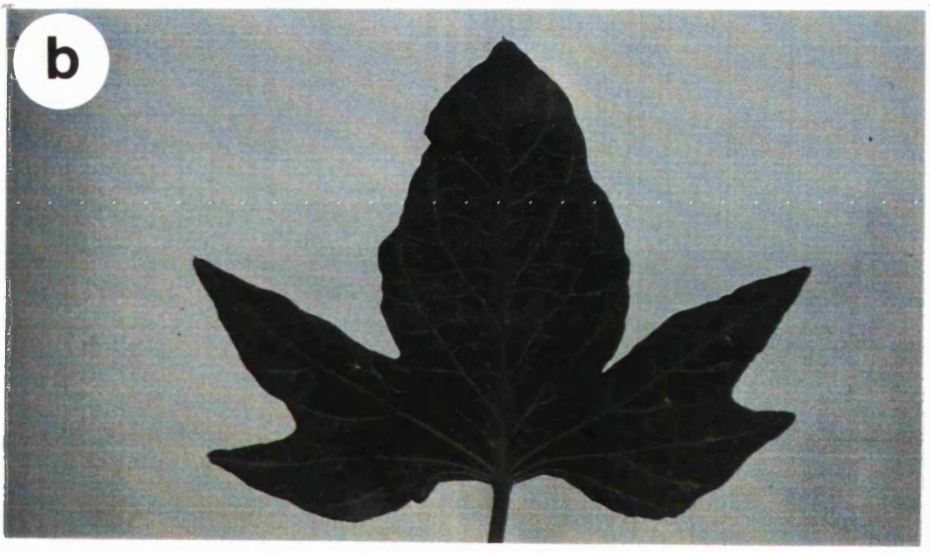
Fig. 17I. Symptoms induced by sweet potato mild mottle virus (SPMMV) in different sweet potato cultivars, of sweet potato, *Ipomoea setosa* and *Nicotiana spp.*

a. Symptoms in sweet potato.

b. Symptoms in sweet potato.

c. Symptoms in *Ipomoea setosa.*

Fig. 17-I



d. Symptoms in *Nicotiana tabacum.*

e. Symptoms in *Nicotiana clevelandii.*

f. Symptoms of severe stunting and distortion in *N. clevelandii.*

Fig·17-I

Fig- 17-II

17II. Particle morphology of SPMMV

a. SPMMV. Partial purified preparation Bar scale represents 300 nm .

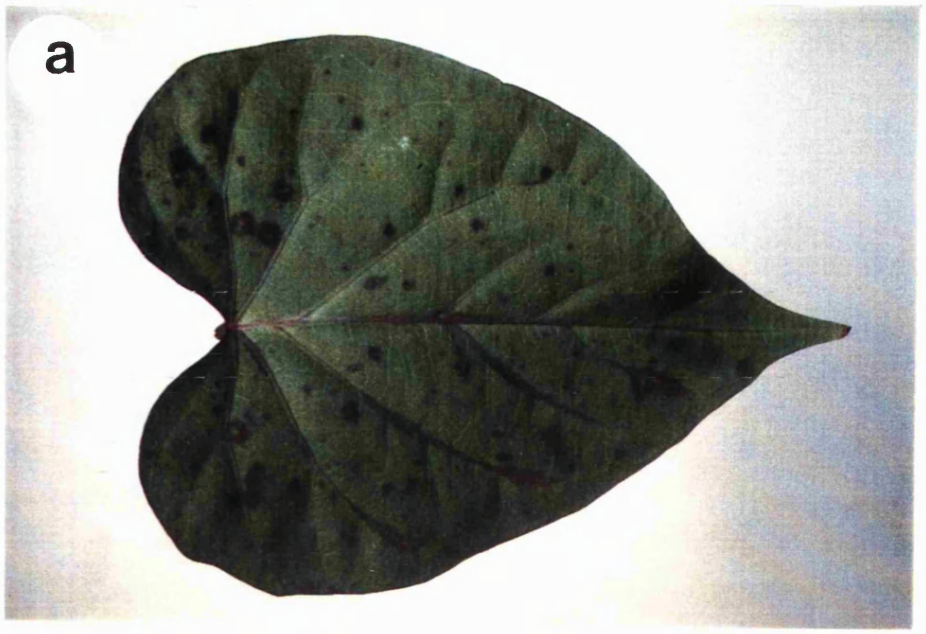
Fig. 18. Symptoms induced by sweet potato featherly mottle virus (SPFMV) in different cultivars of sweet potato.

a. maroon spots and veins.

b. chlorotic spots with maroon margins.

c. maroon vein-banding.

Fig. 18



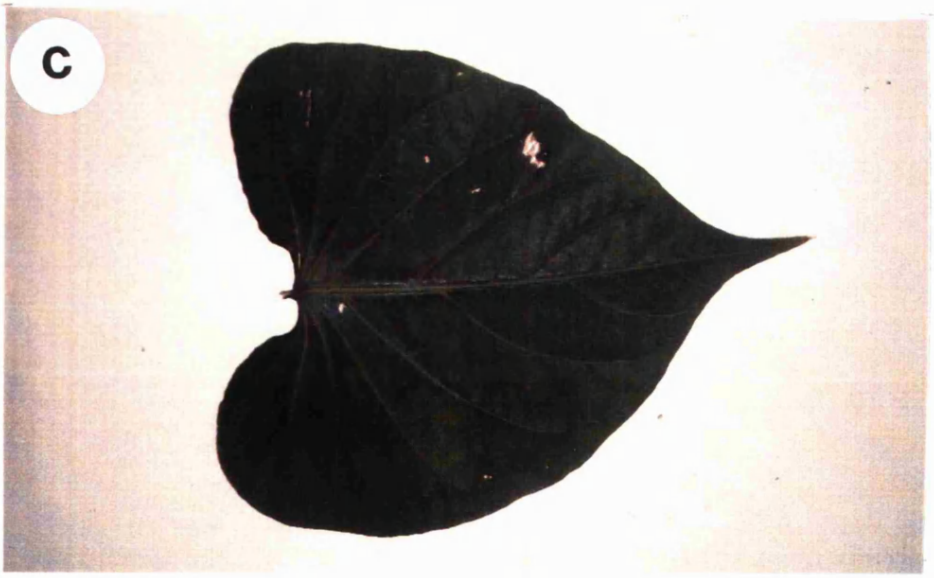
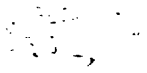


Fig. 18. Symptoms induced by sweet potato featherly mottle virus (SPFMV) in different cultivars of sweet potato.

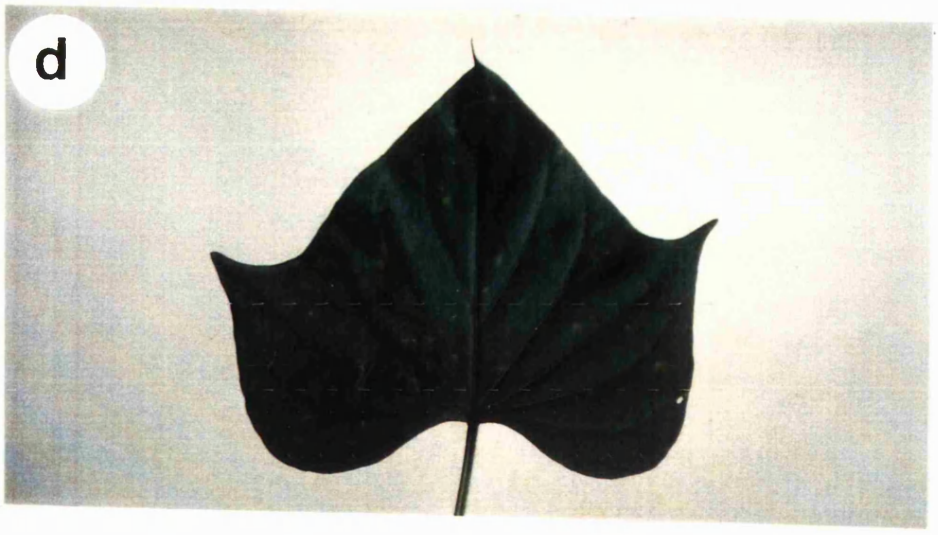


d. few chlorotic spots on leaf (cv. Rose Centennial).

e. numerous chlorotic spots.

f. chlorotic spots developing maroon margins.

Fig-18



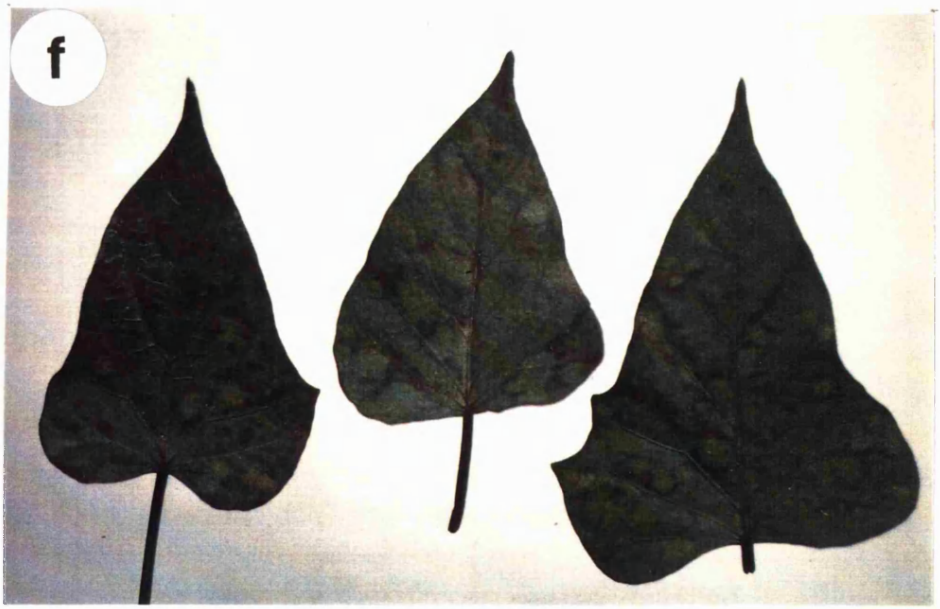


Fig. 19I. Symptoms induced by SPFMV in *Ipomoea setosa* after graft transmission.

a. Initial symptoms of chlorotic spotting followed by vein banding after graft transmission to *I. setosa.*

b. Symptoms of chlorotic vein banding on mature leaf of *I. setosa.*

c. Symptoms of SPFMV infected *Ipomoea nil* after mechanical transmission.

Fig. 19-I

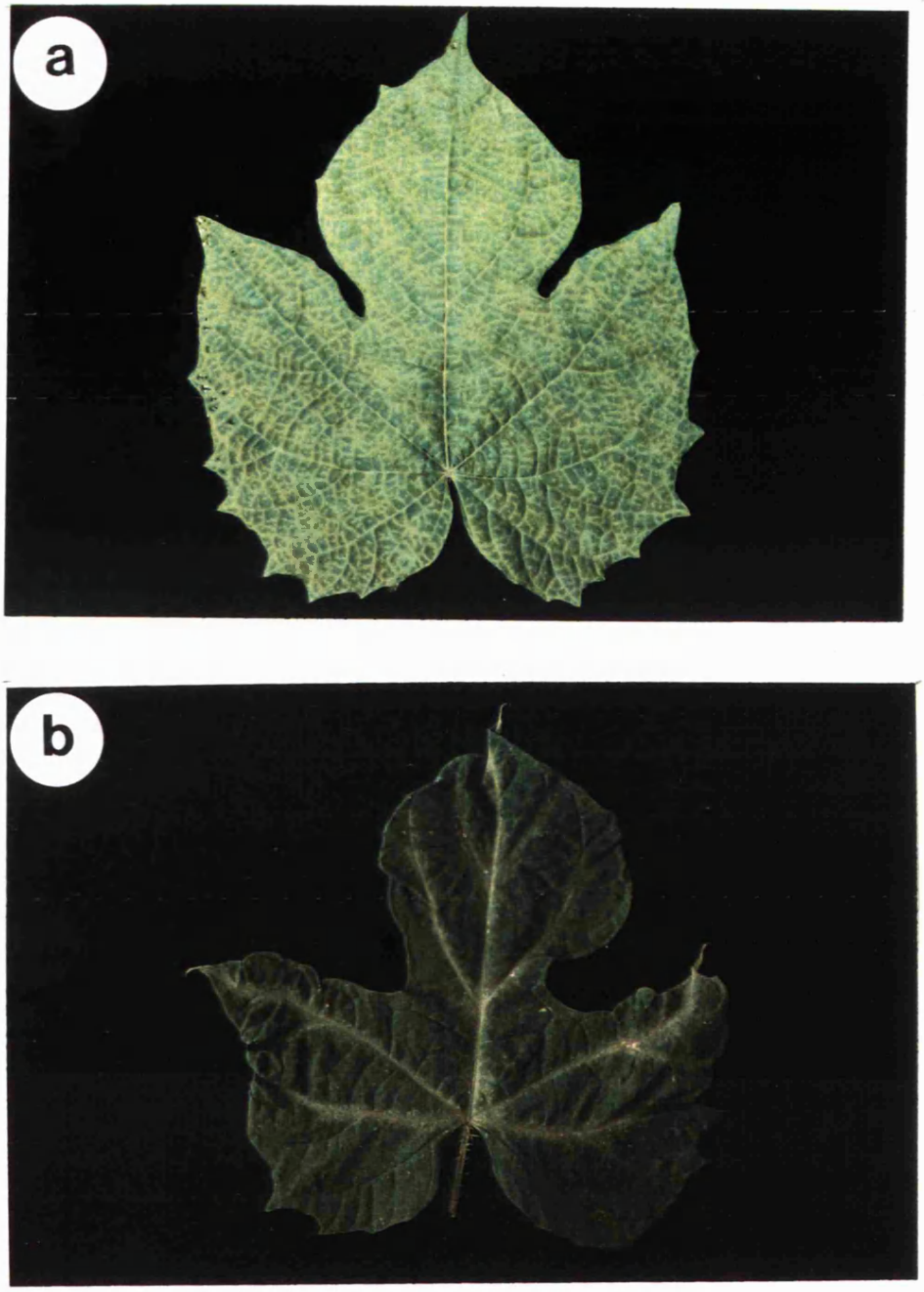
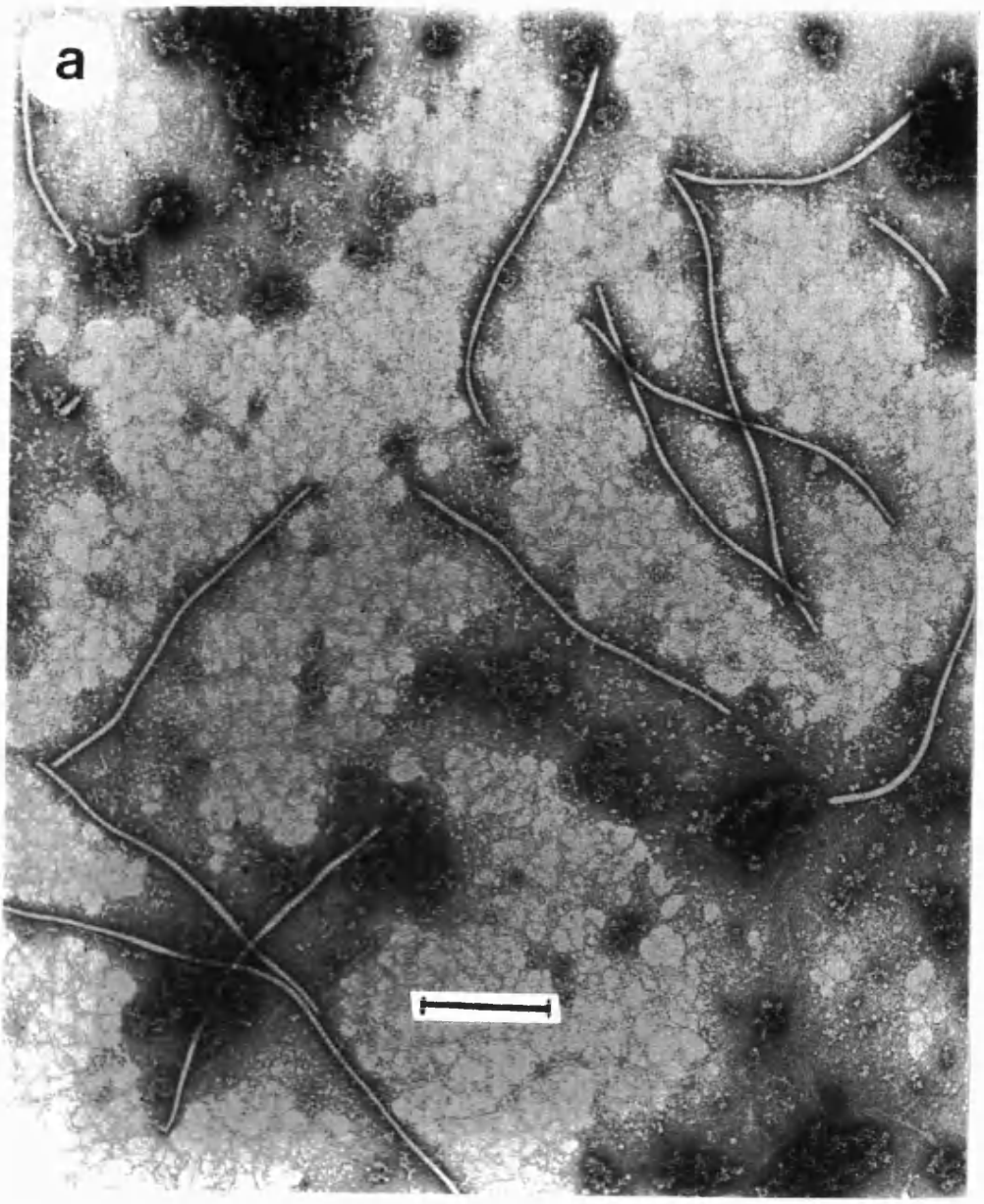


Fig-19-II

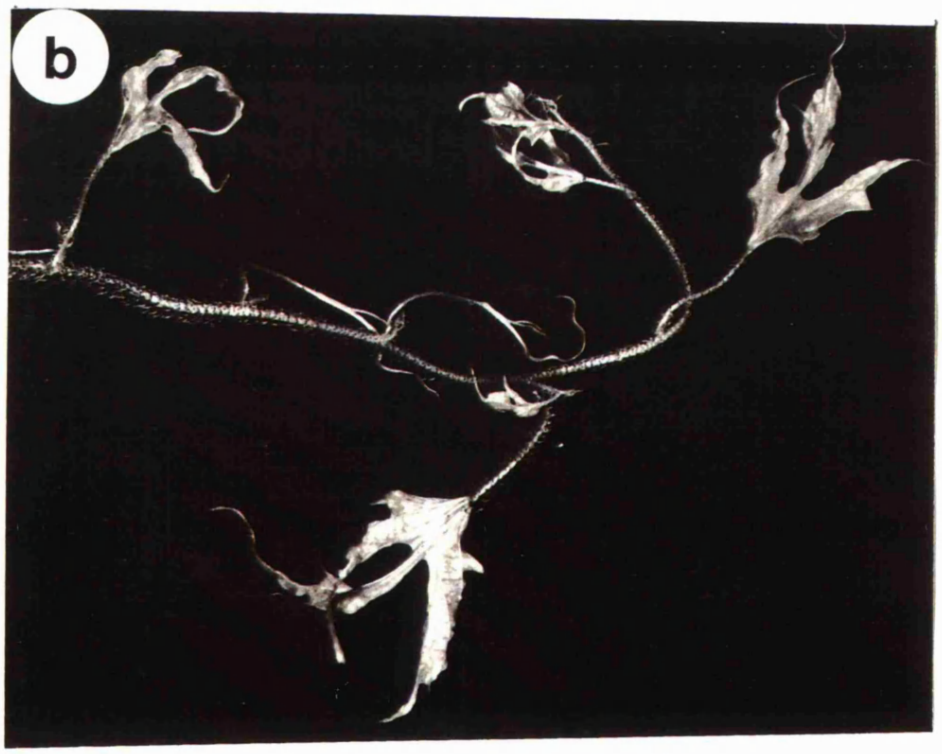


19II. Particle morphology of SPFMV.

a. SPFMV. Particles in leaf extract of infected Ipomoea setosa mounted in 2% neutral potassium phosphotungstate. Bar scale represents 250 nm .

Fig. 20 Symptoms induced by sweet potato mild mottle virus and virus-like agent complex (SPMMV+VLA) in sweet potato and *Ipomoea setosa*.

a. Symptoms in sweet potato.



b. *Ipomoea setosa* showing severe symptoms of shoe-stringing.

c. Symptoms in *Ipomoea setosa* showing chlorosis and severe distortion.

d. Healthy (left) infected (right) *Ipomoea setosa* showing shoe-stringing.

e. Detailed healthy (left) and infected (right) *Ipomoea Setosa.*

Fig·20

3.3.2 EPIDEMIOLOGY

3.3.2.1 Re-infection

Table 17 summarises the results of tests for reinfection of healthy plants by the six viruses (SPFMV, SPMMV, SPLV, SPC-LV, SPCSV, and CMV) at the four experimental sites. The high levels of re-infection at all sites recorded previously (3.3.1.2) are further illustrated in Figs 21-24.

The first assays, made towards the end of the first season in August 1989, indicated that the highest level of re-infection occurred at Kakamega (SPFMV 25%, SPMMV 14%, SPLV 61% and SPC-LV 10%). Relatively high levels of reinfection occurred also in Embu (SPFMV 13%, SPMMV 3%, SPLV 30%). Of special interest was the detection of only SPLV at Katumani (49%) and Muguga (42%).

Assays at the end of the second season in May 1990 showed that levels of infection by three of the viruses (SPFMV, SPCSV, and CMV) was high at Embu (59-73%) and Muguga (55-77%), whereas at Katumani two of the viruses (SPFMV and CMV) occurred at levels of 53-65%; at Kakamega, only the incidence of SPFMV (70%) was high. Although assays for SPC-LV were not made at Embu, it occurred at levels of 22% and 29% at Katumani and Muguga, respectively. SPFMV was detected at all sites in 41-47% of experimental plants.

Tests for reinfection at the end of the third season in November 1990 were not made at Kakamega because plants in the experimental plots had been destroyed by weevils. At the other three sites, there had been high levels of re-infection by SPFMV, SPMMV, SPLV, SPCSV and CMV. Re-infection levels were highest with SPFMV, levels of re-infection for Muguga, Embu and Katumani being 95%, 87% and 79%, respectively. The spread of SPCSV was also high, the relative levels of re-infection at the three sites being 84, 81 and 69%. SPMMV infected 71%, 73% and 60% of

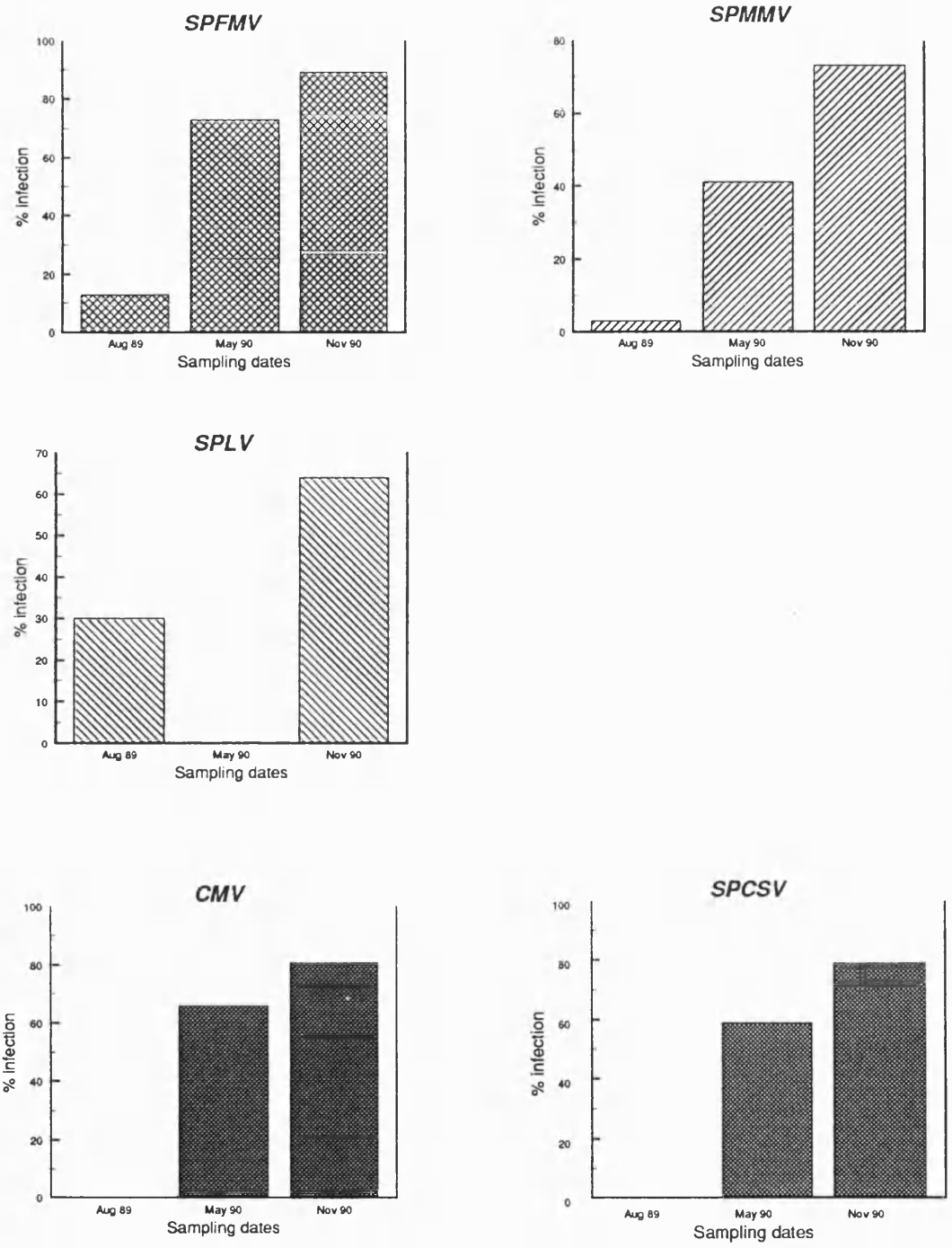
plants at Muguga, Embu and Katumani, respectively, and CMV 84%, 81% and 69%. Re-infection by SPLV, although initially high (see Figs 21-24), was finally detected in 64% and 69% of plants at Embu and Muguga.

Table 17 LEVELS (%) OF RE-INFECTION AT FOUR EXPERIMENTAL LOCATIONS, Aug. 1989 to Nov. 1990.

Assay date	Replicate No.	Embu						Katumani						Muguga						Kakamega					
		F	M	L	C	B	S	F	M	L	C	B	S	F	M	L	C	B	S	F	M	L	C	B	S
August 1989	*R1	10	3	23	0	-	-	0	0	50	0	-	-	0	0	40	0	-	-	33	23	70	7	-	-
	R2	17	7	27	0	-	-	0	0	50	0	-	-	3	0	40	0	-	-	23	7	57	13	-	-
	R3	13	0	40	0	-	-	0	0	47	0	-	-	0	0	50	0	-	-	17	13	57	10	-	-
	Avg.	13	3	30	0	-	-	0	0	49	0	-	-	0	0	43	0	-	-	24	14	61	10	-	-
MAY 1990	R1	63	43	-	-	60	63	53	33	-	20	43	40	70	20	-	43	47	40	90	40	-	60	50	45
	R2	73	40	-	-	60	53	70	47	-	20	60	40	80	60	-	23	63	67	65	45	-	35	55	55
	R3	83	40	-	-	77	60	73	53	-	27	57	57	80	60	-	20	63	57	55	40	-	40	40	40
	Avg.	73	41	-	-	66	59	65	44	-	22	53	46	77	47	-	29	58	55	70	42	-	45	48	47
NOV.. 1990	R1	83	67	53	-	77	73	73	47	60	-	57	60	93	53	60	-	80	63	-	-	-	-	-	-
	R2	93	80	73	-	87	83	80	63	80	-	77	70	93	83	67	-	90	90	-	-	-	-	-	-
	R3	90	73	67	-	80	80	83	70	83	-	73	90	100	77	80	-	83	93	-	-	-	-	-	-
	Avg.	87	73	64	-	81	79	79	60	74	-	69	73	95	71	69	-	84	82	-	-	-	-	-	-

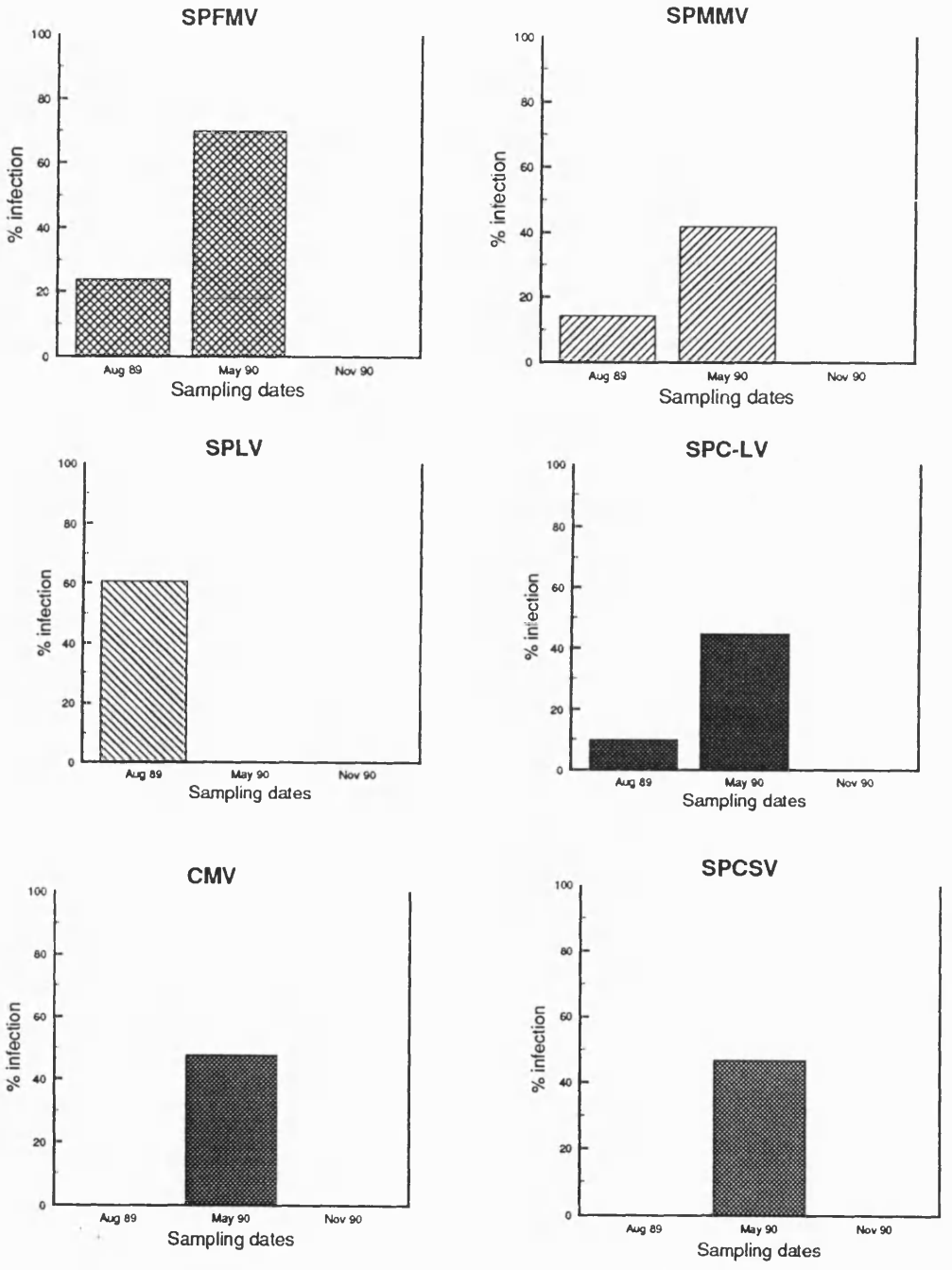
(-) = virus assay not done.
F = SPFMV, M = SPMMV, L = SPLV, C = SPC-LV, B = CMV, S = SPCSV.
$R_{1,2,3}$ = assay rows
Sample size - 90 plants per location.
* R-three blocks, each containing 10 plants per replicate (30 plants).

Fig. 21. LEVELS (%) OF RE-INFECTION AT EMBU



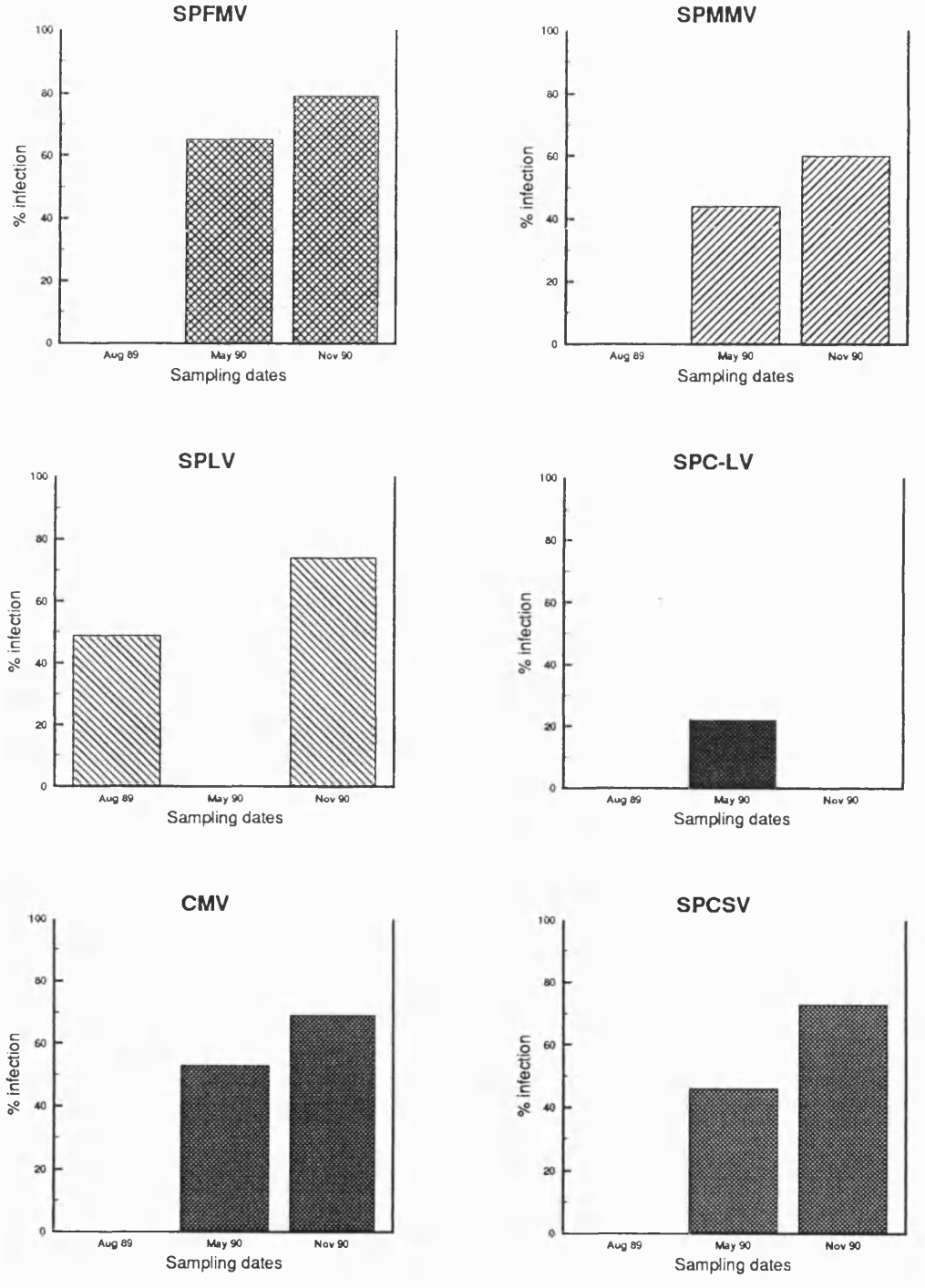
% infection of all 90 plants sampled

Fig. 22. LEVELS (%) OF RE-INFECTION AT KAKAMEGA



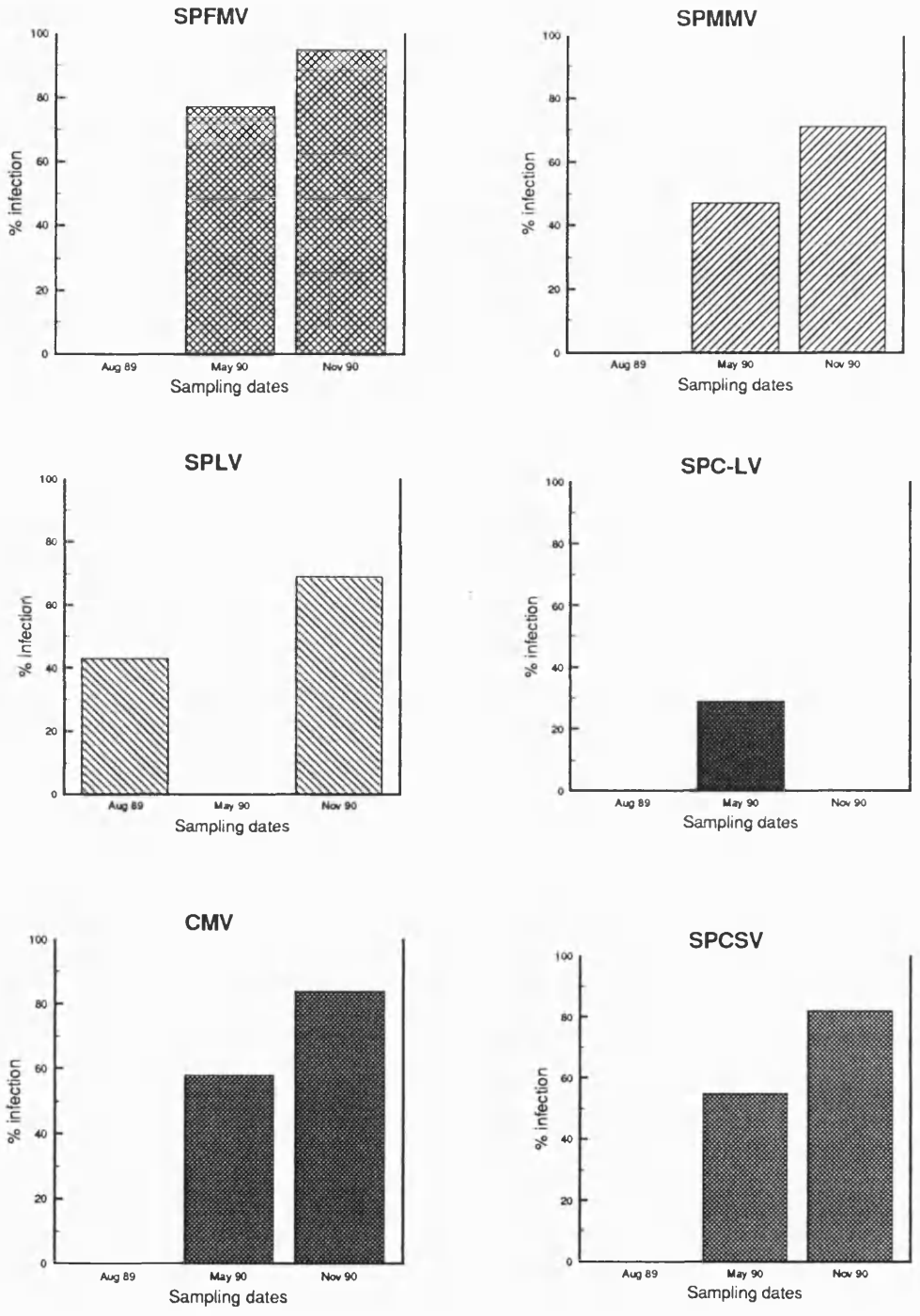
65 of 90 plants sampled

Fig. 23. LEVELS (%) OF RE-INFECTION AT KATUMANI



% Infection of all 90 plants sampled

Fig. 24. LEVELS (%) OF RE-INFECTION AT MUGUGA



% infection of all 90 plants sampled

3.3.2.2 Spread of Sweet Potato Viruses in Relation to Distance from Foci of Infection at Muguga

The results of the experiment to investigate the extent of spread of six viruses (SPFMV, SPMMV, SPLV, SPC-LV, SPCSV, and CMV) in relation to distance from foci of infection at Muguga are given in Table 18, and are further illustrated in Figs 25 to 30. Assays were made in November 1989 (i.e. 7 months after planting) for only four viruses (SPFMV, SPMMV, SPLV, SPC-LV). The results indicate that levels of re-infection in the first season of three viruses (SPFMV, SPMMV, SPC-LV) were very low in all plots, and that the levels of re-infection decreased inversely with the distances from the foci; thus, "near" (1m), "middle" (25 m), and "far" (50 m) plots had on average for the 3 viruses 8%, 7% and 4% infection, respectively. The spread of SPLV was initially greater than the other viruses in all plots, with "near" and "middle" plots having similar levels of infection (54%) which were only slightly higher than that of the "far" plots (38%).

Results of the second test in May 1990, about one year after planting, indicated that on average, 50% of all plots, at all distances from the foci, had become re-infected with the five viruses (SPFMV, SPMMV, SPC-LV, SPCSV, CMV) for which they were tested; the average levels of infection by the five viruses in the "near" "middle" and "far" plots, being 48%, 50% and 44%, respectively. These results indicate that re-infection in the "middle" plots was slightly higher than in "near" plots, this possibly being attributable to sources of infection in two directions; re-infection was less in "far" plots. The extent of spread of individual viruses was generally similar, except for that of SPFMV which was exceptionally high (69%, 76%, 59% for "near" "middle" and "far" plots, respectively); whereas that of the other four viruses ranged between 34-54%, 38-52%, 24-58% in "near" "middle" and "far" plots, respectively.

Results of the last assays, made in November 1990 (18 months after planting), indicated very extensive re-infection in all plots ranging from 68-95%; these results clearly show that, during the last six months, distance had little effect on reducing the spread of SPFMV, SPMMV, SPLV, SPCSV, and CMV. Thus the average for all tested viruses at the three distances, "near", "middle", and "far" plots, indicated levels of infection of 82%. Of individual viruses, the highest rate of re-infection (up to 95% in "near" plots and 91% in "middle" and "far" plots) occurred with SPFMV. Although the spread of SPLV was initially high (November 1989), it was detected finally in 80% of plants, a level of re-infection only slightly higher than that of SPMMV (72%).

Table 18 SPREAD OF SWEET POTATO VIRUSES IN RELATION TO DISTANCE FROM FOCI OF INFECTION AT MUGUGA

Virus	Assay date	Plot No. 1-4 "Near" (1m) No. (%)	Plot No. 5-8 "Middle" (25m) No. (%)	Plot No. 9-12 "Far" (50m) No. (%)	Total No. (%)
SPFMV	Nov.-89	10 - (10)	7 - (7)	1 - (1)	18 - (5)
	May-90	66 - (69)	73 - (76)	57 - (59)	196 - (68)
	Nov.-90	91 - (95)	87 - (91)	87 - (91)	265 - (92)
SPMMV	Nov.-89	5 - (5)	3 - (3)	7 - (7)	15 - (5)
	May-90	33 - (34)	50 - (52)	33 - (34)	116 - (40)
	Nov.-90	67 - (70)	75 - (78)	65 - (68)	207 - (72)
SPLV	Nov.-89	52 - (54)	52 - (54)	36 - (38)	140 - (49)
	Nov.-90	78 - (81)	79 - (82)	76 - (79)	233 - (81)
SPC-LV	Nov.-89	9 - (9)	7 - (7)	5 - (5)	21 - (7)
	May-90	35 - (36)	37 - (39)	23 - (24)	95 - (33)
CMV	May-90	52 - (54)	42 - (44)	56 - (58)	150 - (52)
	Nov.-90	83 - (86)	77 - (80)	86 - (90)	246 - (85)
SPCSV	May-90	45 - (47)	40 - (42)	45 - (47)	130 - (45)
	Nov.-90	75 - (78)	77 - (80)	80 - (83)	232 - (81)

Fig. 25. SPREAD OF SPFMV IN RELATION TO DISTANCE FROM FOCI OF INFECTION AT MUGUGA

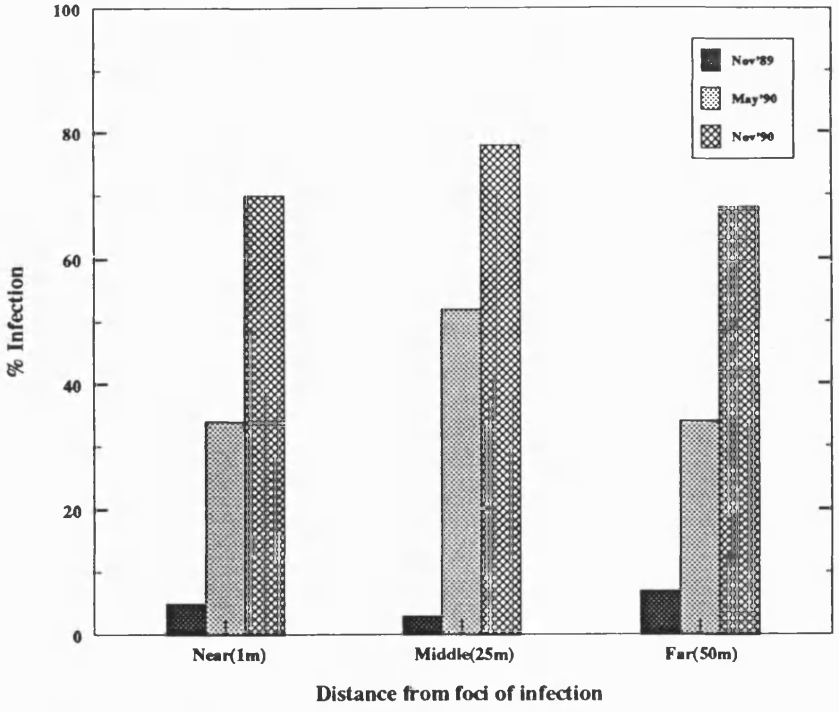


Fig. 26. SPREAD OF SPMMV IN RELATION TO DISTANCE FROM FOCI OF INFECTION AT MUGUGA

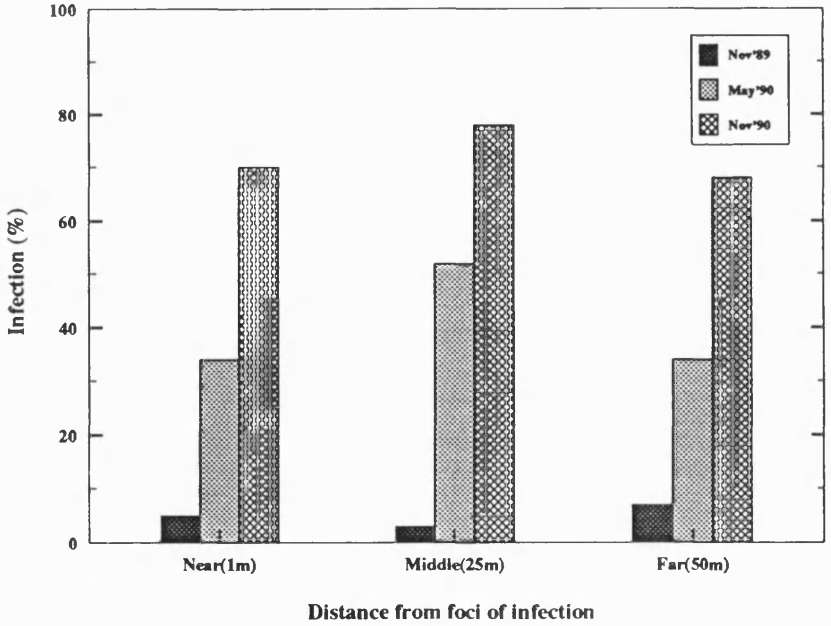


Fig. 27. SPREAD OF SPLV IN RELATION TO DISTANCE FROM FOCI OF INFECTION AT MUGUGA

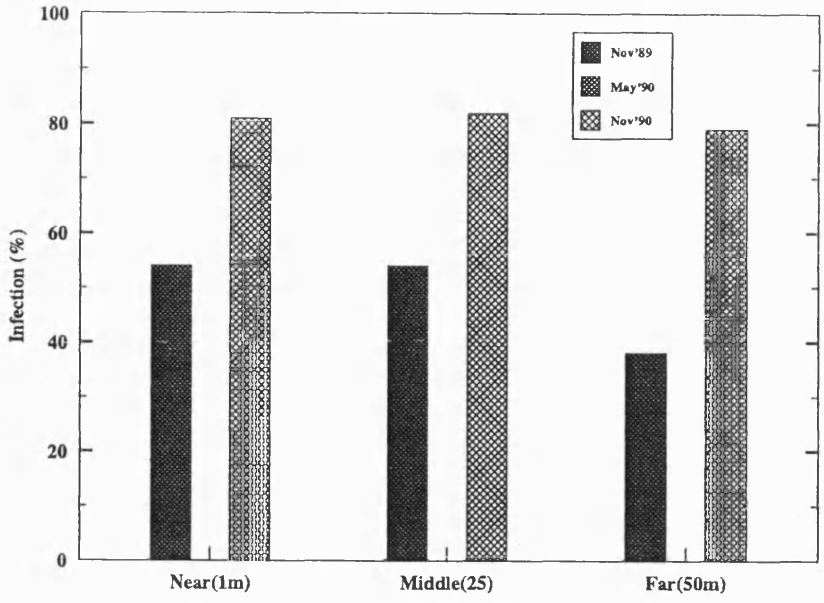


Fig. 28. SPREAD OF SPC-LV IN RELATION TO DISTANCE FROM FOCI OF INFECTION AT MUGUGA

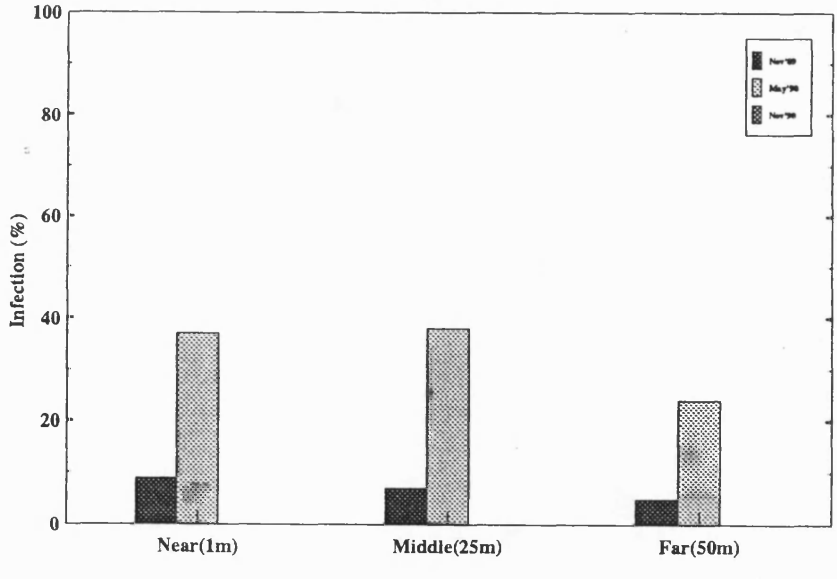


Fig. 29. SPREAD OF CMV IN RELATION TO DISTANCE FROM FOCI OF INFECTION AT MUGUGA

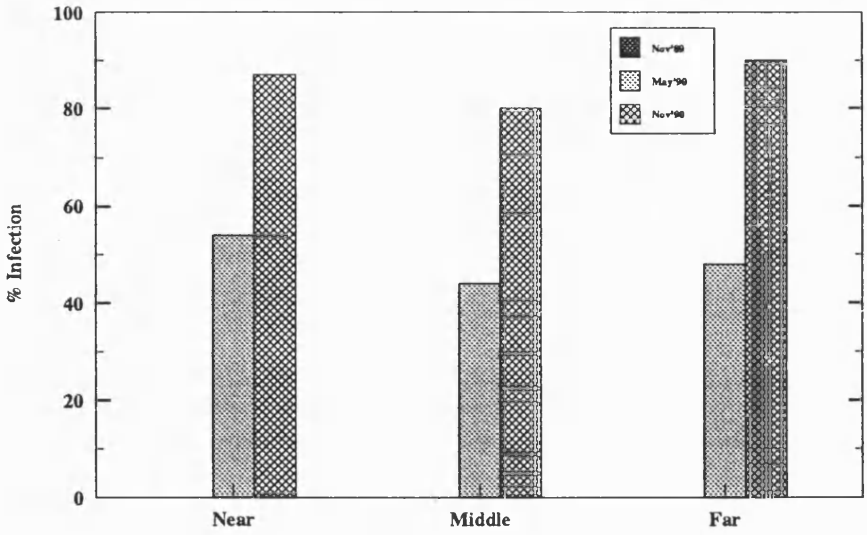
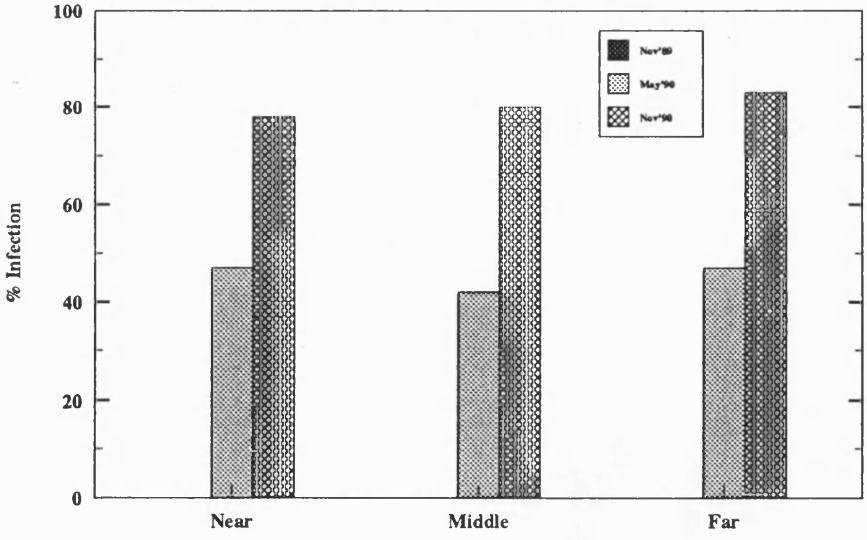


Fig. 30. SPREAD OF SPCSV IN RELATION TO DISTANCE FROM FOCI OF INFECTION AT MUGUGA



3.3.2.3 Spread of Sweet Potato Viruses at Muguga in Relation to Prevailing Southerly Wind Direction

The spread of sweet potato viruses (SPFMV, SPMMV, SPLV, SPC-LV, SPCSV, and CMV) from foci of infection into "near" (1m) "middle" (25m) and "distant" (50m) experimental plots during three seasons is summarized in Table 19; that for individual viruses is presented in Figs 31 to 36 respectively.

The prevailing southerly wind had a slight overall effect on the pattern of virus spread. Although little effect was detected at the end of the first season (May, 1989), more upwind spread of viruses occurred during the second and third seasons. Thus at the end of the second season (May 1990), the average levels of infection for SPFMV, SPMMV, SPC-LV, SPCSV and CMV in plots North, East, South and West of the infection source (i.e. control plots) were 54, 50, 42, 46%, respectively. Comparable values for SPFMV, SPMMV, SPCSV and CMV at the end of the third section (Nov. 1990) were 84, 84, 79, and 82% respectively.

There were, however, notable differences in the spread of individual viruses during the second and third seasons. Thus, lowest levels of infection by SPMMV, SPCSV, and SPC-LV occurred in plots to the south of the infection source, and those of SPFMV and CMV were only a little higher than those in the western plots. At the end of the third season, southern plots had the lowest levels of infection by all viruses except those infected by SPLV in which infection was a little higher than western plots.

Table 19 SPREAD OF SWEET POTATO VIRUSES AT MUGUGA IN RELATION TO PREVAILING SOUTHERLY WIND DIRECTION

VIRUS	Assay dates	Plots (*) to the North			East			South			West		
		N (1) **	M (5)	F (9)	N (2)	M (6)	F (10)	N (3)	M (7)	F (11)	N (4)	M (8)	F (12)
SPFMV	11/89	4	2	0	3	1	0	1	2	1	2	2	0
	5/90	17	22	16	17	18	15	18	16	13	14	17	13
	11/90	23	23	20	23	22	23	21	22	21	24	20	23
SPMMV	11/89	1	13	17	3	0	2	0	0	4	1	3	1
	5/90	0	13	20	4	12	9	3	10	8	13	15	7
	11/90	0	9	16	14	21	16	15	18	16	21	16	17
SPLV	11/89	10	14	8	15	14	13	16	11	9	11	13	6
	5/90	-	-	-	-	-	-	-	-	-	-	-	-
	11/90	20	21	17	18	20	23	20	18	20	20	20	16
SPC-LV	11/89	3	2	0	3	1	1	1	3	3	2	1	1
	5/90	13	12	6	9	11	7	6	8	3	7	6	7
	11/90	-	-	-	-	-	-	-	-	-	-	-	-
SPCSV	11/89	-	-	-	-	-	-	-	-	-	-	-	-
	5/90	13	10	11	12	11	11	9	8	13	11	11	10
	11/90	22	19	19	18	20	20	18	18	20	17	20	21
CMV	11/89	-	-	-	-	-	-	-	-	-	-	-	-
	5/90	16	10	13	14	13	16	12	9	14	10	10	13
	11/90	23	19	23	20	20	24	19	20	18	21	18	21

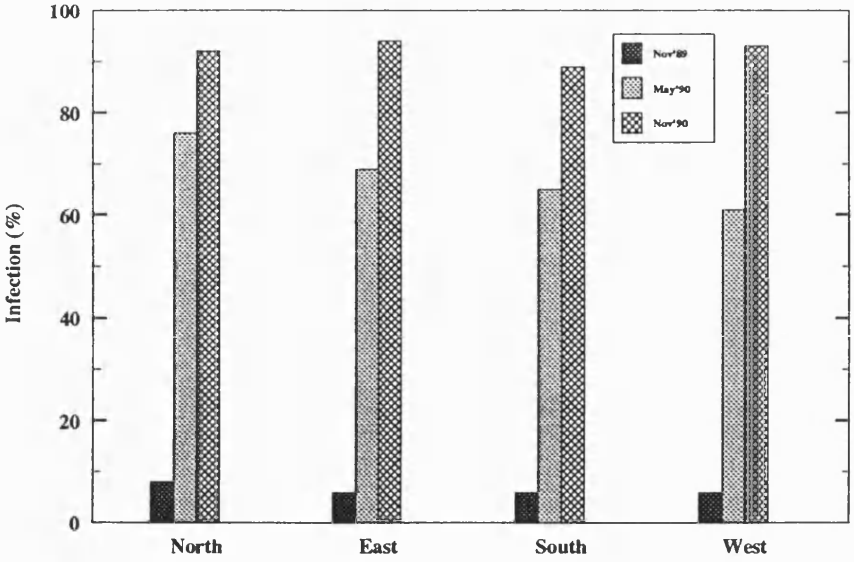
N= "Near" (1m), M= "Middle" (25m), F= "Far" (50m)

* Each plot contained 24 plants

** Numbers in parentheses are plot numbers indicated in the experimental plan (Fig. 8).

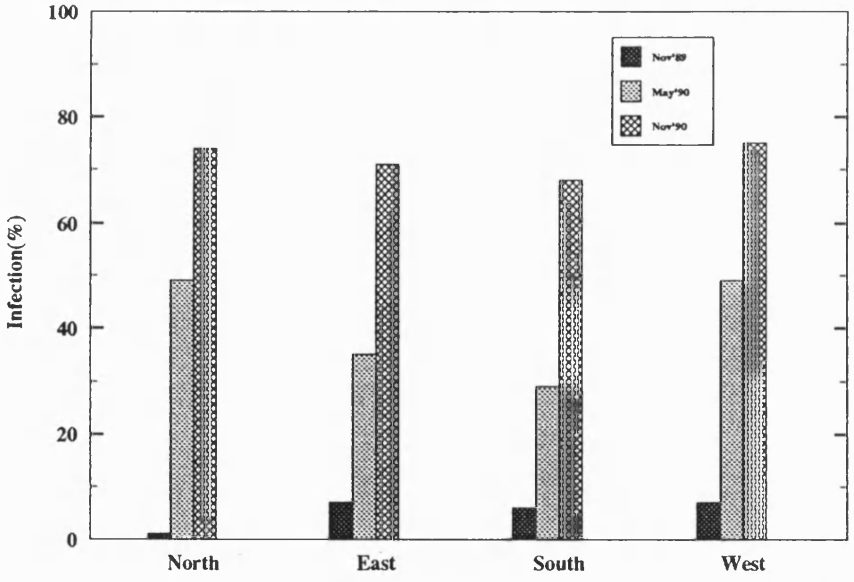
(-) virus assay not done.

Fig. 31. PREVAILING SOUTHERLY WIND DIRECTION AND SPREAD OF SPFMV AT MUGUGA



Direction from the central focus of infection

Fig. 32. PREVAILING SOUTHERLY WIND DIRECTION AND SPREAD OF SPMMV AT MUGUGA



Direction from the central focus of infection

Fig. 33. PREVAILING SOUTHERLY WIND DIRECTION AND SPREAD OF SPLV AT MUGUGA

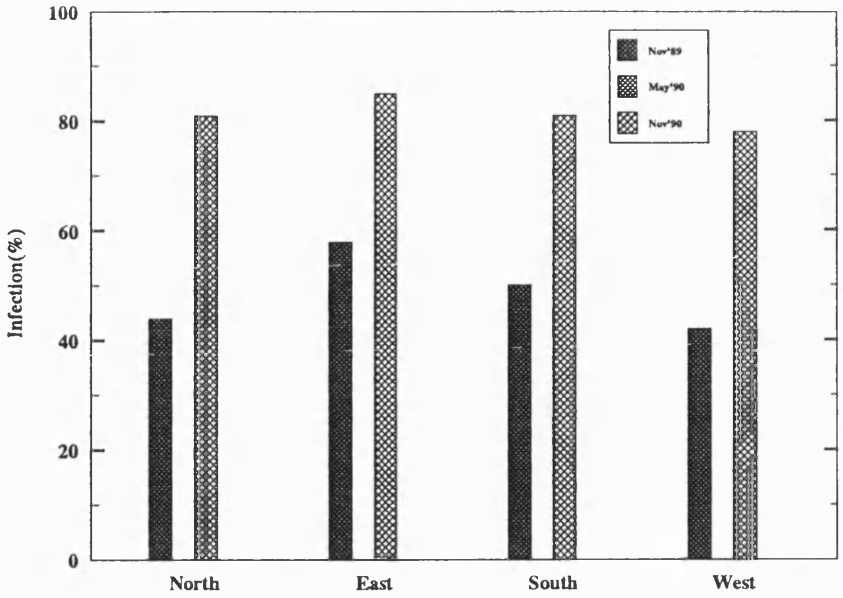


Fig. 34. PREVAILING SOUTHERLY WIND DIRECTION AND SPREAD OF SPC-LV AT MUGUGA

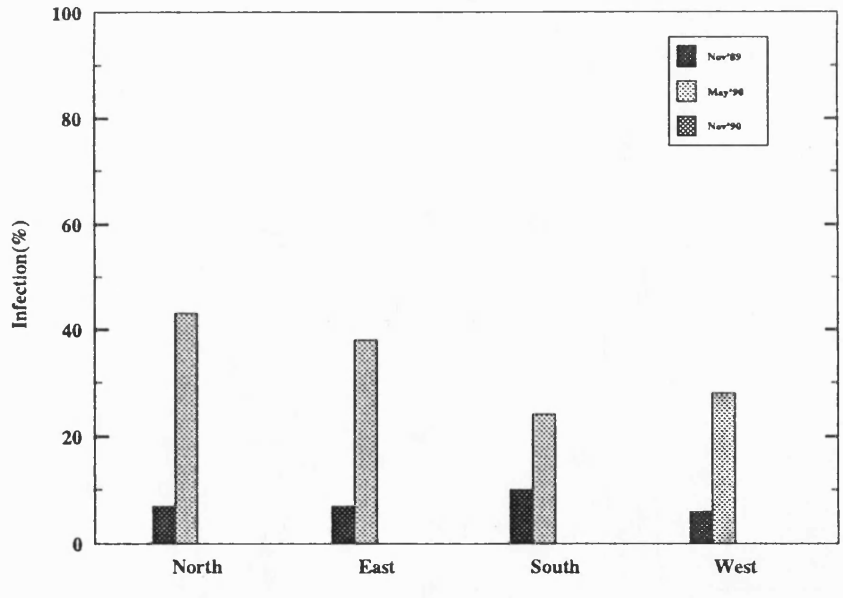


Fig. 35. PREVAILING SOUTHERLY WIND DIRECTION AND SPREAD OF CMV AT MUGUGA

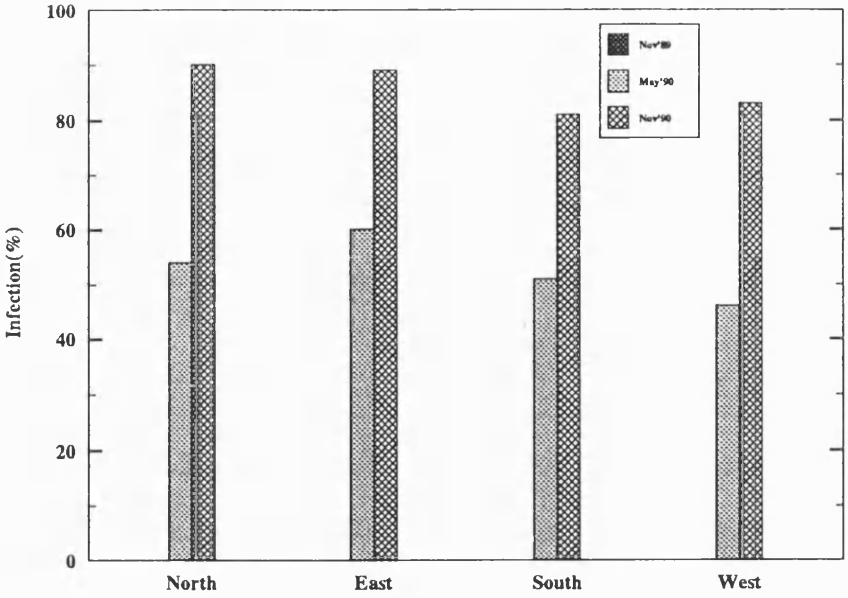
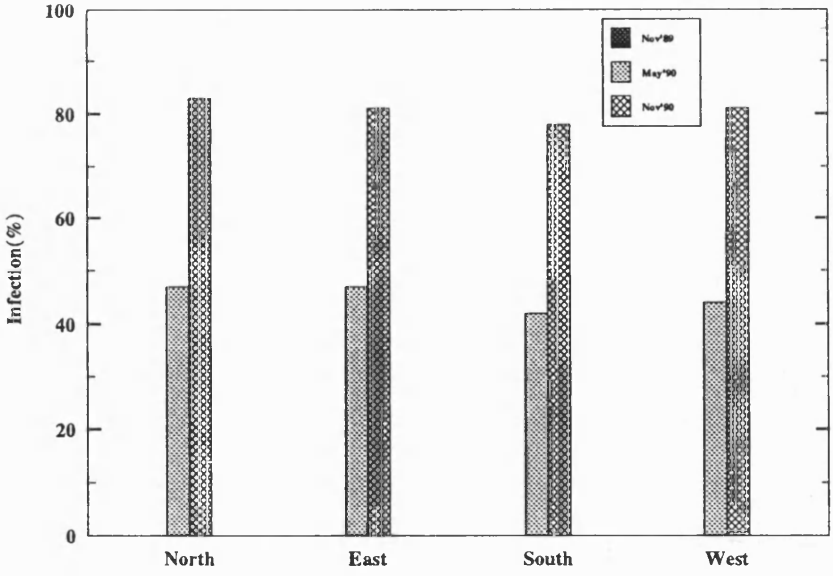


Fig. 36. PREVAILING SOUTHERLY WIND DIRECTION AND SPREAD OF SPCSV AT MUGUGA



3.3.3 Aphid and Whitefly Trapping

3.3.3.1 Trapping of Aphid Species

The aphid species and their numbers trapped at the four sites (Embu, Kakamega, Katumani, and Muguga) from December 1989 to November 1990, are given in Table 20 and Figs 37,39, 41, 43 respectively. Unfortunately, due to trap failures, no insects were collected from May-July at Kakamega and from April-July at Katumani. Of the 39 aphid species caught in the yellow water traps, only one could not be identified. Of the 39 trapped species, 28 were recorded in Embu, 25 in Kakamega, 20 in Katumani, and 29 in Muguga; 13 species were trapped at all four sites, although in differing numbers, and included four known vectors of SPFMV and many of CMV (Kennedy, Day and Eastop, 1962).

The trapping records show (Figs 37, 39, 41, 43) that large populations of aphids occur in February and March at Kakamega, Katumani and Muguga and in December at Embu, and also in June at Muguga and Embu. The species that were especially abundant were *Aphis craccivora, Aphis fabae, Aphis gossypii* and *Aphis spiraecola*; other species that occurred commonly were *Brevicoryne brassicae, Lipaphis erysimi, Myzus persicae, Rhopalosiphum maidis, Sitobion* spp., *Tetraneura nigriabdominalis* and *Toxoptera citricidus*.

Most crops in Kenya, including those at the four sites in Embu, Katumani, Muguga and Kakamega, are usually planted during the period of long rains (Table 21 and Figs 38, 40, 42, 44) of March to May. However, the cropping reason extends to July/August when rainfall gradually decreases as crops mature and are harvested; in June, usually the peak month of the season, most crops have reached physiological maturity but are not ready for harvest. There is also a second and short cropping season from October to December which follows the short rains during which short season crops like some beans, potatoes and early maturing sweet potato cultivars like

Mwezi-tatu are planted. December, January and February are the driest months of the year, and most crops (especially annual crops), are not then grown; however, sweet potato stock plants have to be maintained in the field for harvesting during the next cropping season. The major climate and soil conditions, and the main crops for the four experimental locations are given in Appendix 1. Kakamega differs slightly from the other three sites in that the annual total rainfall is more evenly distributed (Fig 40) throughout the year and it also has the highest evapotranspiration rate (Appendix I). Katumani is also different in that it is located in the semi-arid region of marginal farming or cropping systems. At the four sites, relatively high aphid populations generally coincided with the main rainfall and cropping season from March to August and is well illustrated in Figs 37 and 38 for Embu, 39 and 40 for Kakamega, 41 and 42 for Katumani and 43 & 44 for Muguga; 43% and 68% of the total aphid populations occurred during the season at Embu and Muguga respectively, with June being the peak month and coinciding with the time of crop maturity.

The species known to be vectors of SPFMV (see 3.3.3.2) were always a significant proportion of the total caught throughout the year at each site, and at Embu and Muguga exceeded 50% of those caught in May, June and July (Figs 45-48). At least 12 of the trapped species are also known to be efficient vectors of CMV (see 3.3.3.2).

Table 20. APHID SPECIES CAUGHT IN YELLOW WATER TRAPS IN FOUR DIFFERENT LOCATIONS IN KENYA (Dec. 1989-Nov. 1990).

	Total catches at				
Aphid species	Embu	Kakamega	Katumani	Muguga	Total
Aphis craccivora *#	90	62	5	199	356
Aphis fabae #	77	53	17	90	237
Aphis gossypii *#	216	46	15	89	366
Aphis nerii	2	7	1	6	16
Aphis pseudocardui	2	2	-	-	4
Aphis spiraecola	387	345	67	108	907
Brachycaudus helichrysi #	4	-	1	2	7
Brachycaudus rumexicolens	4	-	-	9	13
Brevicoryne brassicae #	35	10	3	12	60
Cimara cupressi	-	-	-	8	8
Dysaphis foeniculus #	6	-	-	-	6
Eucarazzia elegans	-	-	-	2	2
Gesicu lucifiga	2	-	-	-	2
Hallaphis Malawi	-	1	1	-	2
Hayhurstia atriplicis	-	-	-	8	8
Hyperomyzus lactucae	5	1	-	2	8
Hysteroneura setariae	6	1	-	6	13
Hyperomyzus carduellinus	1	-	-	-	1
Kugegania ageni	-	-	1	1	2
Lipaphis erysimi *#	21	32	1	29	83
Macrosiphum euphorbiae *#	-	-	-	1	1

Table 20. (Continued)

	Total catches at				
Aphid species	Embu	Kakamega	Katumani	Muguga	Total
Melanaphis sacchari	1	9	1	-	11
Myzus ornatus #	-	2	-	21	23
*Myzus persicae #**	46	62	13	26	147
Nyalopterus pruni	-	-	1	-	1
Pentalonia nigronervosa	1	7	1	-	9
Rhopalosiphum maidis #	29	2	1	16	48
Rhopalosiphum padi #	5	2	2	10	29
Rhopalosiphum rufiabdominalis	-	2	-	4	6
Saltusaphis scirpus	-	-	-	1	1
Schoutedenia lutea	7	1	-	-	8
Sitobion spp.	15	2	7	9	33
Tetraneura nigriabdominalis	12	9	1	9	31
Toxoptera aurantii	1	-	-	-	1
Toxoptera citricidus	35	6	3	17	61
Toxoptera odinae	1	3	-	3	7
Uroleucon compositae	1	3	-	9	13
Uroleucon sonchi	1	-	1	9	11
Un-identified	-	1	-	2	3

* Known vectors of SPFMV

Known vectors of CMV

Table 21. MEAN MONTHLY RAINFALL (mm) FOR EMBU, KAKAMEGA, KATUMANI AND MUGUGA DURING THE INSECT MONITORING PERIOD

(DEC. 1989 - NOV. 1990)

Station	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Embu (Fig 41)	58.3	89.9	16.6	223.7	429.8	180.0	20.7	27.4	22.5	71.7	217.6	307.7
Kakamega (Fig 42)	158.5	116.2	173.6	230.1	227.5	175.3	74.4	87.4	429.9	136.4	119.3	178.4
Katumani (Fig 43)	129.5	42.8	23.2	217.3	250.7	65.9	5.1	0.0	3.4	0.0	48.8	208.5
Muguga (Fig 44)	84.0	45.0	61.7	234.9	259.4	267.4	19.8	3.8	14.3	1.6	86.7	137.9

Data from KARI Meteorological Department Annual Report 1989/1990.

Fig. 37. CATCHES OF KNOWN VECTOR AND OTHER APHID SPECIES AT EMBU

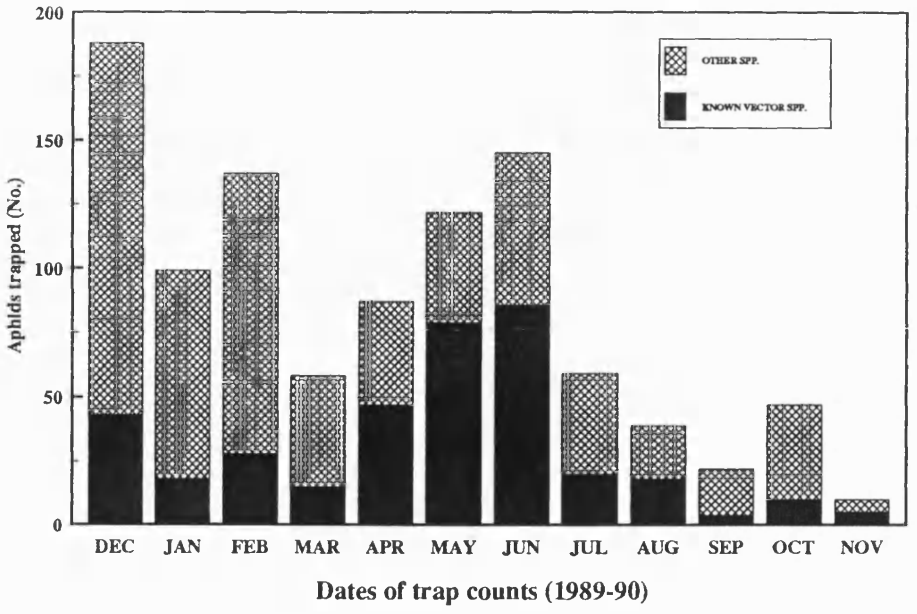
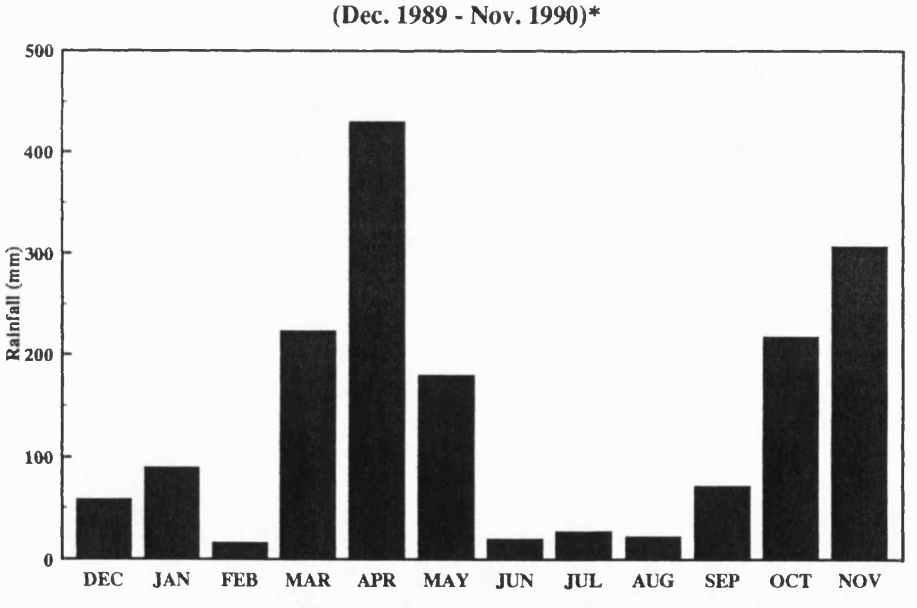
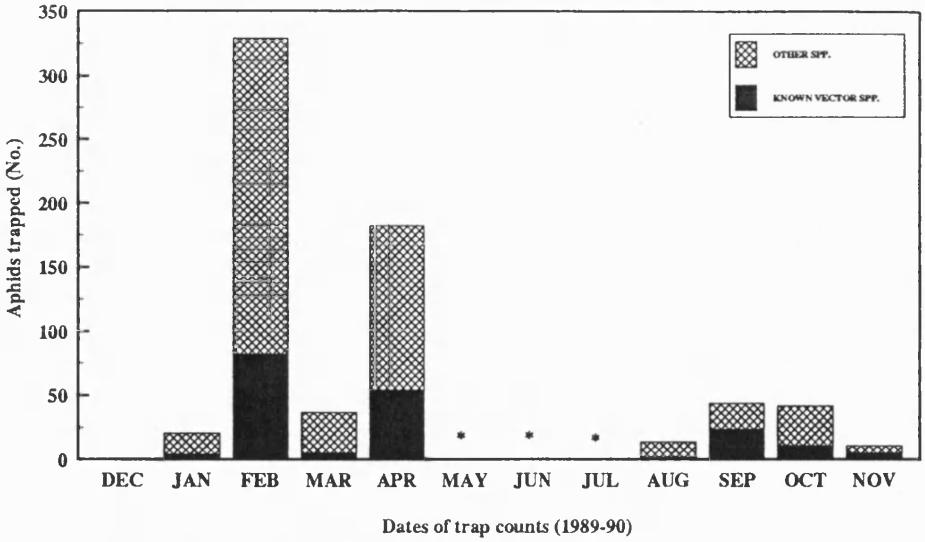


Fig. 38. MEAN MONTHLY RAINFALL (mm) FOR EMBU DURING THE INSECT MONITORING PERIOD (Dec. 1989 - Nov. 1990)*



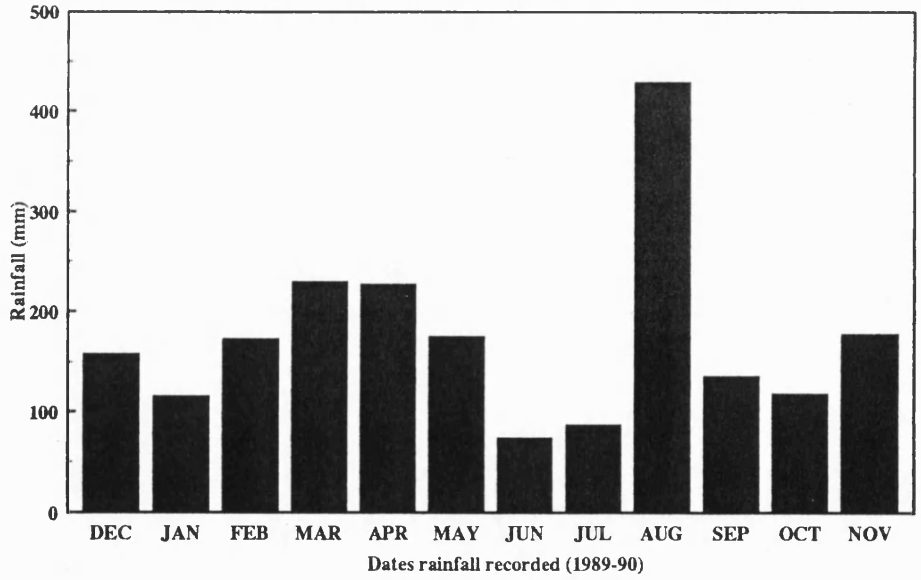
* Data from KARI Meteorological Department Annual Report, 1989/90

Fig. 39. CATCHES OF KNOWN VECTOR AND OTHER APHID SPECIES AT KAKAMEGA



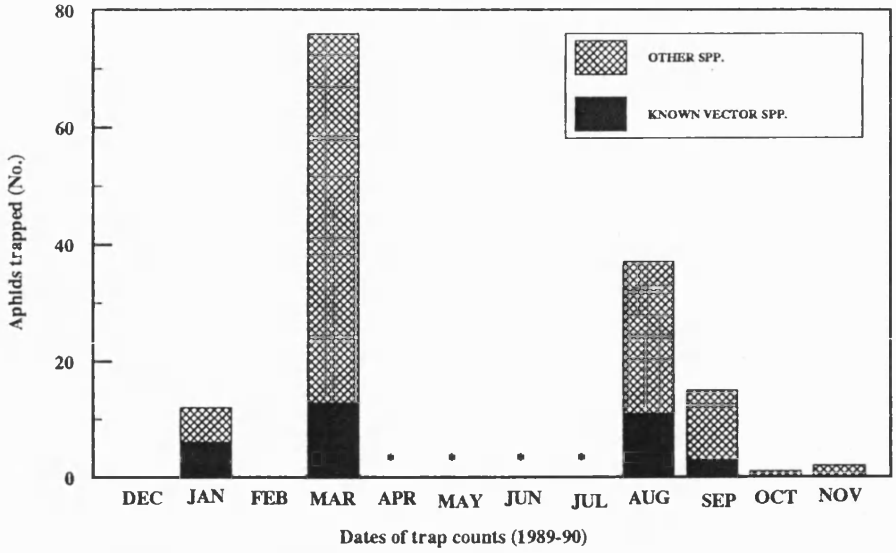
* No samples due to trap failures

Fig. 40. MEAN MONTHLY RAINFALL (mm) FOR KAKAMEGA DURING THE INSECT MONITORING PERIOD (Dec. 1989 - Nov. 1990)*



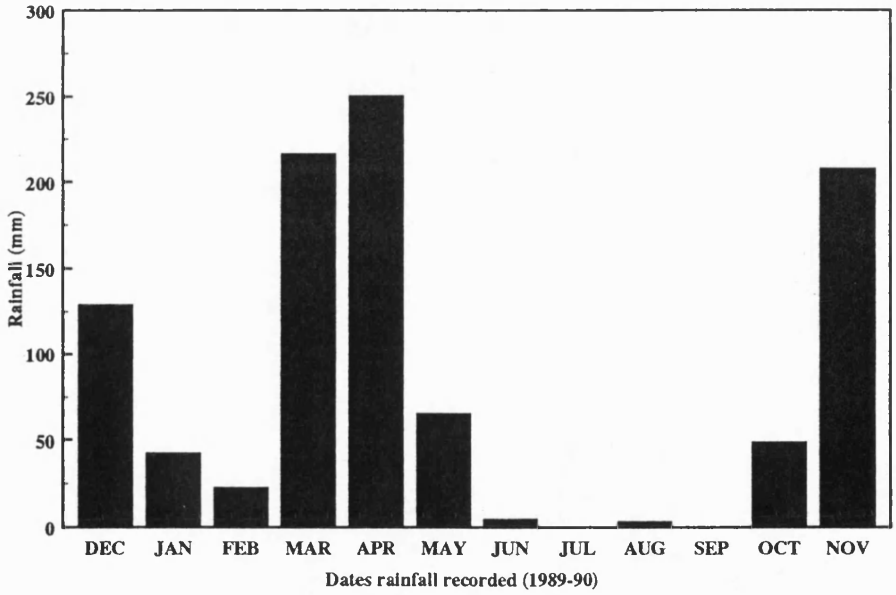
* Data from KARI Meteorological Department Annual Report, 1989/90

Fig. 41. CATCHES OF KNOWN VECTOR AND OTHER APHID SPECIES AT KATUMANI



* No samples due to trap failures

Fig. 42. MEAN MONTHLY RAINFALL (mm) FOR KATUMANI DURING THE INSECT MONITORING PERIOD (Dec. 1989 - Nov. 1990)*



* Data from KARI Meteorological Department Annual Report, 1989/90

Fig. 43. CATCHES OF KNOWN VECTOR AND OTHER APHID SPECIES AT MUGUGA

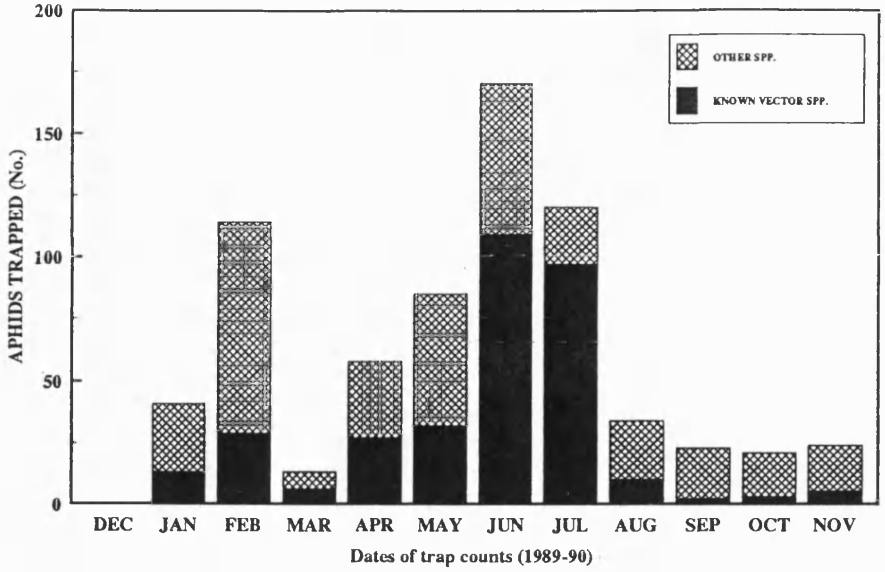
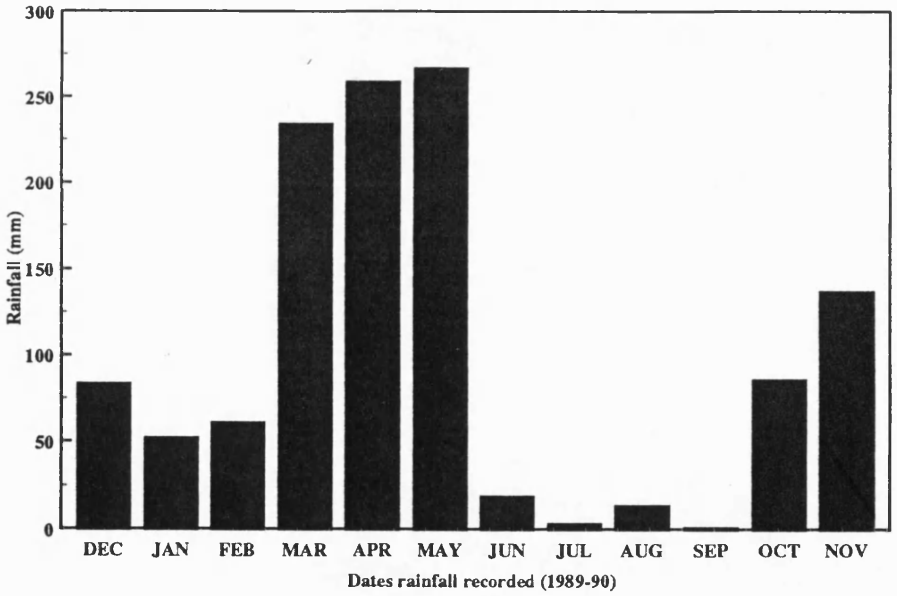


Fig. 44. MEAN MONTHLY RAINFALL (mm) FOR MUGUGA DURING THE INSECT MONITORING PERIOD (Dec. 1989 - Nov. 1990)*



* Data from KARI Meteorological Department Annual Report, 1989/90

3.3.3.2 Catches of Known Virus Vector Aphid Species

Of the 39 aphid species trapped (Table 20), the following five are known to be vectors of SPFMV: *Aphis craccivora* (Moyer and Kennedy, 1978), *Aphis gossypii* (Stubbs and McLean, 1958), *Lipaphis erysimi* (Moyer and Kennedy, 1978), *Myzus persicae* (Stubbs and McLean, 1958) and *Macrosiphum euphorbiae* (Hildebrand and Smith, 1958). All were trapped at all four experimental sites, except *M. euphorbiae* which was recorded only at Muguga. The occurrence and distribution of the four main aphid vector species of SPFMV are shown in Figs 45-48. At least 12 of the species are also known to be efficient vectors of CMV (Kennedy, Day and Eastop, 1962); these are *Aphis craccivora, A. fabae, A. gossypii, Brachycaudus helichrysi, Brevicoryne brassicae, Dysaphis foeniculus, Lipaphis erysimi, Macrosiphum euphorbiae, Myzus ornatus, M. persicae, Rhopalosiphum maidis, and R. padi.* All occurred in fairly high populations. Results also indicated *Aphis craccivora* and *Aphis gossypii* were the predominant species at Embu and Muguga, while at Katumani and Kakamega these and *Myzus persicae* were the most predominant species.

Fig. 45. CATCHES OF FOUR KNOWN APHID VECTOR SPECIES OF SPFMV AT EMBU

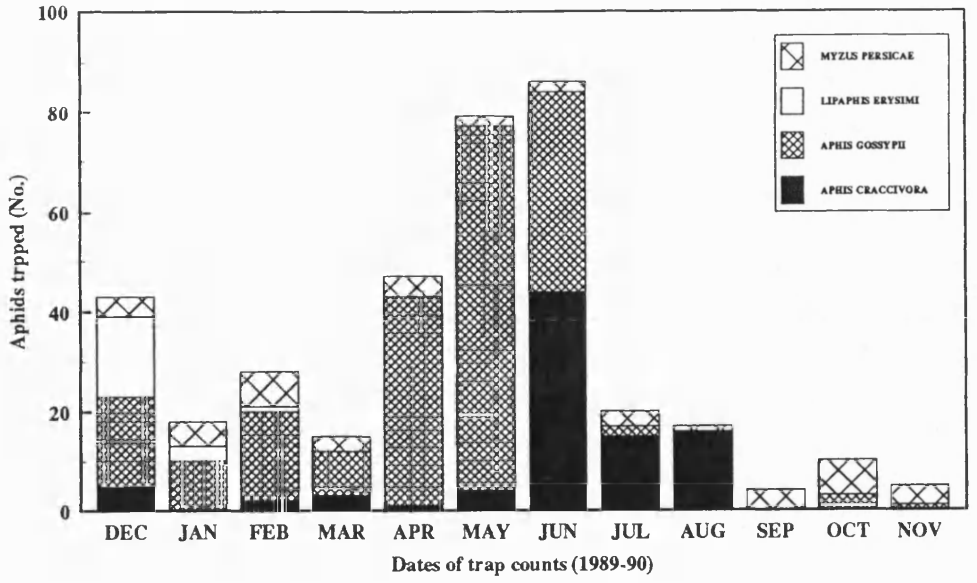
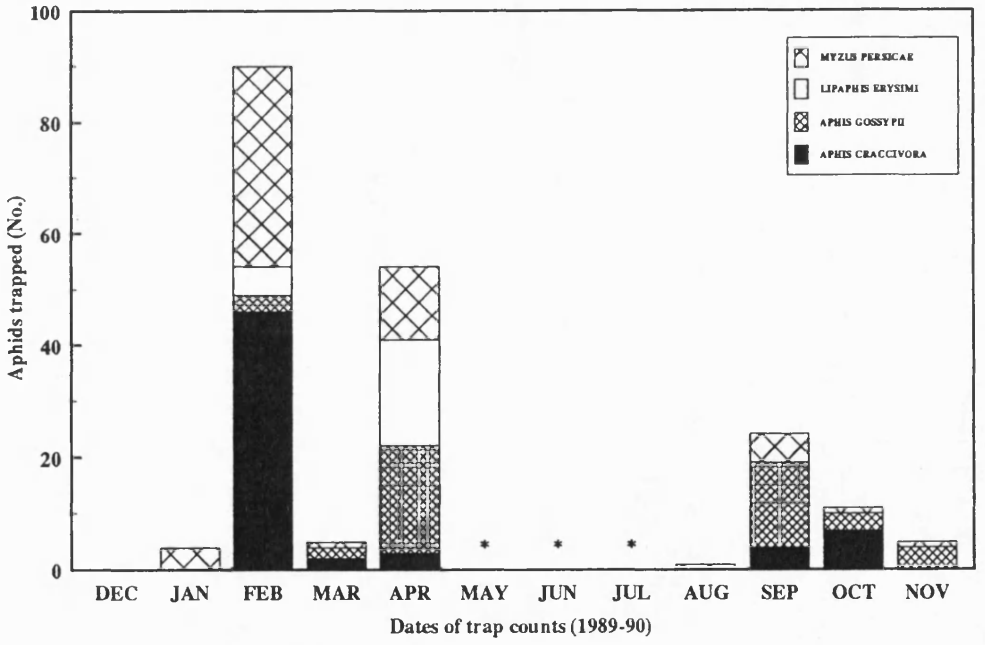
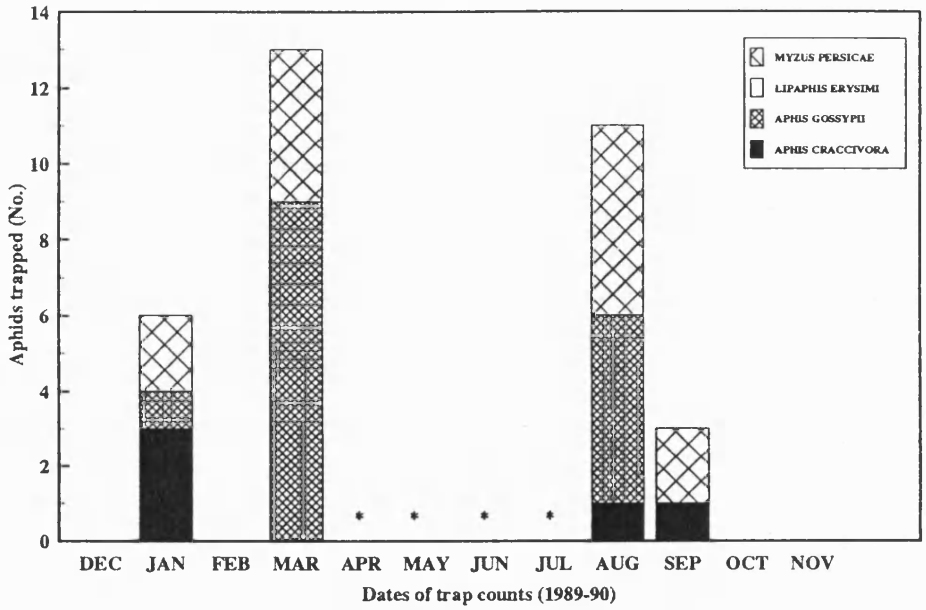


Fig. 46. CATCHES OF FOUR KNOWN APHID VECTOR SPECIES OF SPFMV AT KAKAMEGA



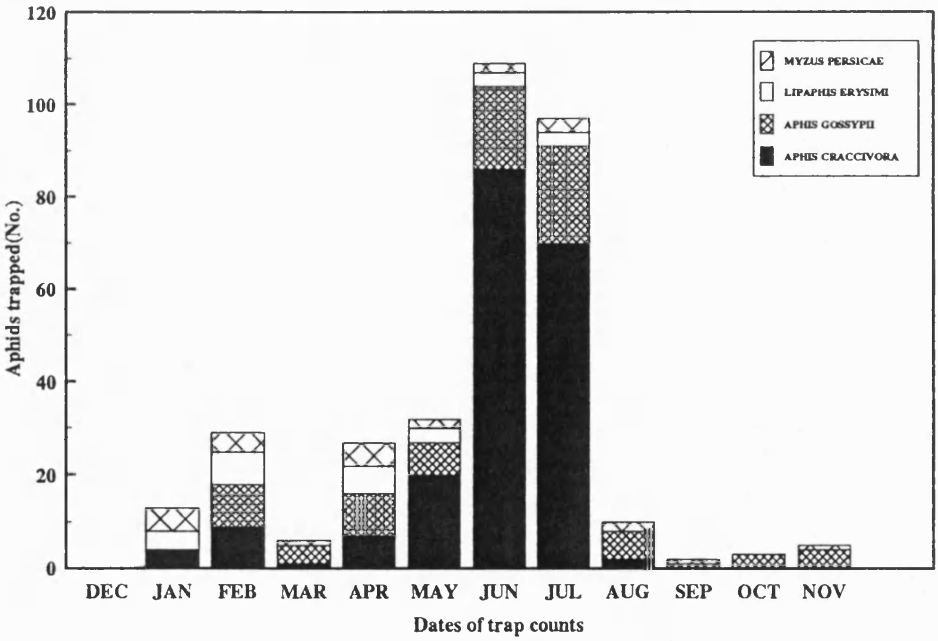
* No samples due to trap failure

Fig. 47. CATCHES OF FOUR KNOWN APHID VECTOR SPECIES OF SPFMV AT KATUMANI



* No samples due to trap failure

Fig. 48. CATCHES OF FOUR KNOWN APHID VECTOR SPECIES OF SPFMV AT MUGUGA



3.3.3.3 Whitefly Catches by Use of 3 Metre Sticky Yellow Column Traps

The number of whiteflies caught at four sites (Embu, Kakamega, Katumani, and Muguga) from Dec. 1989 - Nov. 1990 are given in Table 22, and the results are illustrated in Figs 49, 50, 51, 52, respectively. Unfortunately, due to trap failures, no data were collected from April to July in Katumani. Although it was not possible to specifically identify the adult whiteflies, for which the larval stage is necessary, most were probably *Bemisia tabaci* (Dr Lincoln Fishpool, personal communication). The whiteflies were caught at 50, 100, 150, 200, and 250 cm above soil level. An appraisal of the results presented in Table 22 shows that whitefly catches were, generally, higher nearer the ground and decreased inversely with height of trapping. This trend seemed more pronounced at Embu than the other sites and showed slight seasonal variations. A further appraisal of the results (Figs 49-52) indicated that there were two periods when whitefly population were relatively high the major peak, when the highest numbers were trapped, occurred February/March and a second, relatively smaller, but important, "peak" occurred in or about October. The whitefly numbers were relatively low during the period of long rains in the months of April to July (see Table 21, and Figs 38, 40, 42, 44); this is the relatively cool season and the main cropping season in Kenya.

Table 22. WHITEFLIES CAUGHT (Na) ON STICKY YELLOW COLUMN TRAPS (DEC. 1989 - NOV. 1990)

	DATES OF TRAP COUNTS *												
Experimental site	Trap level **	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV
Embu	1	25	62	188	74	15	2	11	6	48	48	29	19
	2	29	57	65	42	9	0	3	6	20	22	16	11
	3	11	19	28	26	7	2	3	2	9	8	7	13
	4	13	11	16	11	3	1	4	1	9	5	6	4
	5	7	16	6	10	0	0	1	2	1	1	0	0
	Total	105	165	303	163	34	5	22	17	87	84	58	47
Kakamega	1	6	21	52	18	21	18	13	14	19	12	23	22
	2	12	24	46	15	28	9	11	9	14	17	20	15
	3	9	22	33	15	28	17	7	11	19	8	22	10
	4	15	18	33	19	18	16	7	3	22	17	17	13
	5	12	13	23	18	15	7	8	7	14	11	10	11
	Total	54	98	187	85	110	67	47	44	88	65	92	71
Katumani	1	12	16	36	36					3	2	14	13
	2	8	25	27	24					3	2	13	4
	3	6	27	12	24					3	0	18	7
	4	10	16	21	28					2	0	6	14
	5	8	19	13	33					5	0	5	10
	Total	44	103	109	145	***	***	***	***	16	4	56	48

Table 22. (continued)

	DATES OF TRAP COUNTS *											
Experimental site	Trap level **	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV
Muguga	1	19	20	36	35	15	8	14	15	20	16	13	15
	2	17	26	26	25	14	7	11	11	15	13	13	10
	3	21	24	22	23	14	8	6	10	14	13	10	13
	4	20	22	19	20	12	5	4	14	14	12	9	12
	5	18	20	18	19	13	3	4	9	10	5	8	6
	Total	95	112	121	122	68	31	39	59	73	59	53	56

Table 22. (continued)

* Monthly counts from Dec. 1989- Nov. 1990.

** Trap levels 1-5 represent, respectively, 50, 100, 150, 200, and 250 cm above soil level.

*** No data due to trap failures.

Fig. 49. WHITEFLY CATCHES AT EMBU (1989-90)

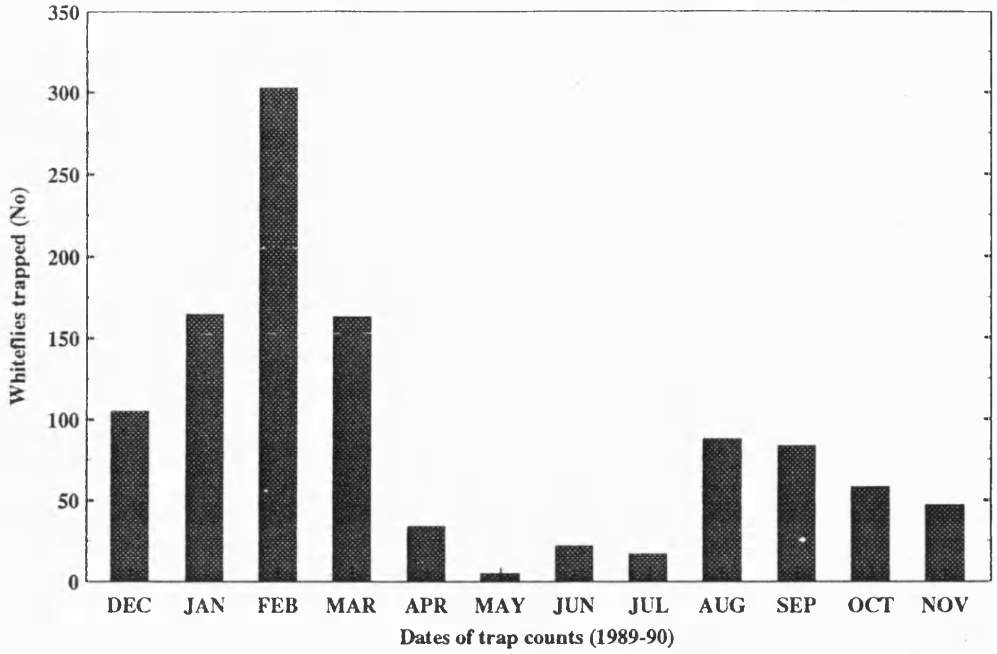


Fig. 50. WHITEFLY CATCHES AT KAKAMEGA (1989-90)

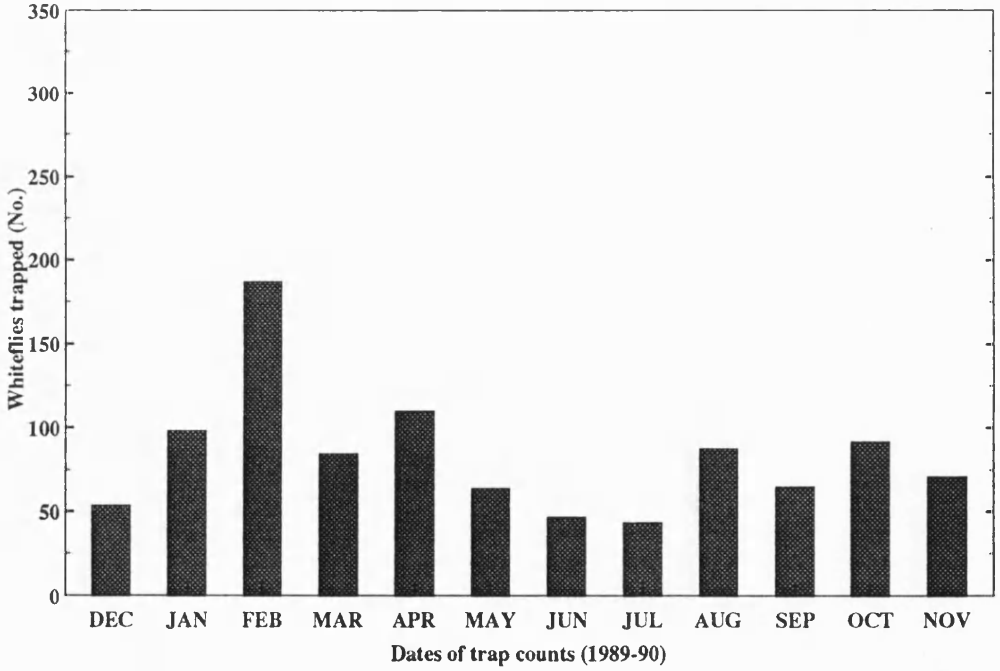
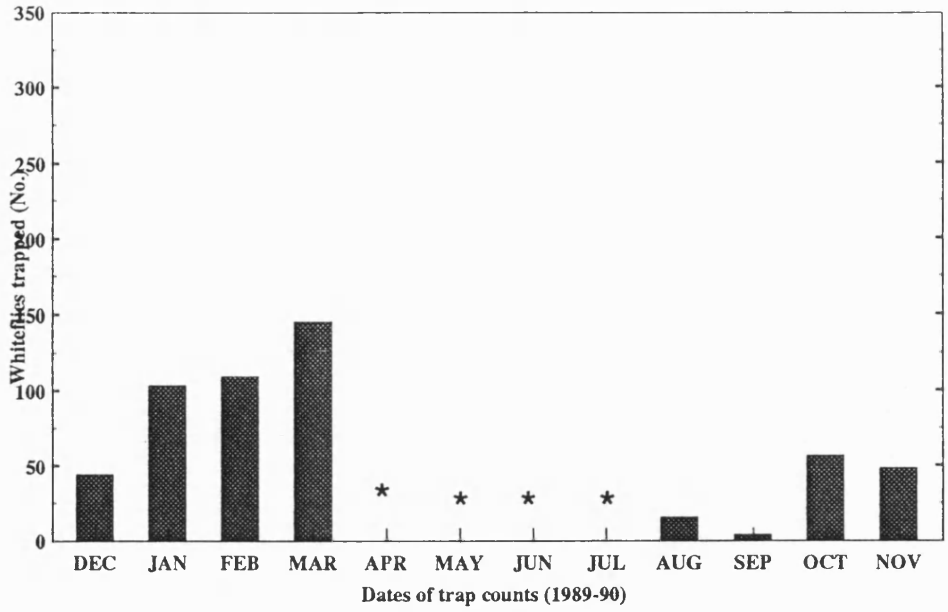
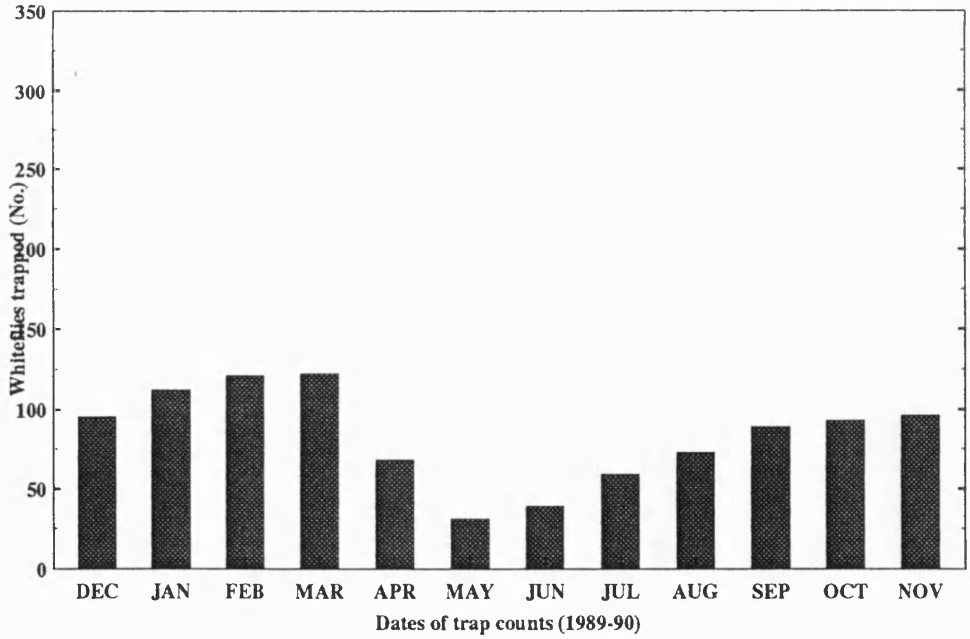


Fig. 51. WHITEFLY CATCHES AT KATUMANI (1989-90)



* No sampling due to trap failures

Fig. 52. WHITEFLY CATCHES AT MUGUGA (1989-90)



3.3.3.4 Whitefly Catches in Yellow Water Traps (January-November 1990)

The numbers of whiteflies caught in the yellow water traps at Embu, Kakamega, Katumani and Muguga are given in Table 23. Unfortunately, due to trap failures, it was not possible to monitor whitefly populations throughout the year at the last three locations. Available data, however, suggest that whitefly populations are higher in February and March and, to a somewhat lesser extent, from August to October, but very low during other months. These results are similar to those obtained using sticky column traps (3.3.3.3).

Table 23. WHITEFLIES CAUGHT (NO.) IN YELLOW WATER TRAPS (JAN.-NOV. 1990)

Trap catches in 1990	Trap Locations			
	Embu	Kakamega	Katumani	Muguga
January	-*	25	5	-
February	337**	23	9	18
March	240	12	5	4
April	15	-	-	-
May	1	-	-	-
June	1	-	-	-
July	8	-	-	-
August	60	10	-	-
September	67	6	1	-
October	28	25	7	-
November	43	2	2	-

* - no data collected.

** Total monthly counts.

3.4 DISCUSSION

During surveys in 1989 and 1990 for viruses infecting sweet potatoes, many farmers indicated the need to increase sweet potato production in Kenya but recognised the major constraints of lack of sufficient land and cultivars with adequate pest and disease resistance, and marketing difficulties (Table 9). It was also observed that individual farmers grew relatively few cultivars, planting stock of which had often been originally obtained from neighbours and retained for many years. The survey also showed that there was an extremely high incidence of virus infection in sweet potatoes in most locations, especially of mixed infections of upto seven viruses. Although farmers selected the best available planting material, they did not practice roguing so that the 10-20% infections introduced as primary and latent infections of the viruses, became important foci for infection and spread during the crop growing season. The survey also showed that positive selection of apparently healthy plants for planting stocks, if further supported by roguing could be effective in controlling sweet potato viruses as indicated by the case of NARC-Katumani KARI, where the lowest incidence of field infection was observed.

Identification of viruses in samples collected during the survey showed that diagnosis by observation at field symptoms alone is impractical, as symptom development is probably influenced by environmental and climatic conditions. An example of this was at Kisii where, in surveys during the rainy season, very few plants were found with symptoms; however, when field samples were carefully tested, many were found to be virus infected. This, perhaps, indicates the need to rogue stocks either very early or late in the season when crops are under stress and symptoms are more likely to be apparent or, better still, to use rapid and sensitive serological methods for virus detection and identification.

Of the 11 viruses and the VLA known to infect sweet potatoes (see section

3.1), seven viruses were detected in Kenya by the use of NCM-ELISA (Table 10). Of these, SPRSV occurred only in two of the six Provinces and at very low incidences (9-10%). SPC-LV did not occur at the Coast of Kenya, but did so elsewhere at relatively low incidences (9-29%). The other viruses occurred prevalently throughout Kenya where sweet potatoes are grown (Table 11). The occurrence of some viruses in complexes (Table 12) was a common feature at all four experimental locations and was correlated with the frequent presence of very severe symptoms and serious degeneration of stocks. It also emphasises the difficulties that would probably be encountered in the need to breed plants for resistance to several viruses.

The partial characterisation of selected virus isolates indicated that those of SPC-LV, SPMMV and SPFMV in Kenya are typical of those described elsewhere (e.g. Clark and Moyer, 1988; Hollings *et al.*, 1976; Atkey and Brunt, 1987). However, more detailed studies on an wider range of isolates from different regions of Kenya are necessary to determine whether isolates differ greatly in virulence or other properties. Of particular interest was the detection during a late stage of the investigations of a severe disease of sweet potatoes which is probably caused by SPMMV in complex with an as yet uncharacterised virus-like agent. This situation is similar to that in Nigeria, Israel and possibly also Taiwan, in which a severe sweet potato disease is known to be induced by the synergistic interaction of SPFMV and a whitefly transmitted, but non-mechanically transmissible, virus-like agent (Hahn *et al.*, 1981; Moyer and Salazar, 1989). However, intensive investigations are now required to determine whether a second agent or pathogen is also involved in Kenya and, if so, to what extent it resembles that occurring in Nigeria and Israel.

Unfortunately, shortage of time also precluded investigations on Kenyan isolates of other viruses (especially SPLV, CMV and SPCSV) which were detected during the surveys by NCM-ELISA, but have yet to be isolated and compared with isolates

occurring elsewhere (Moyer and Salazar, 1989). Further studies with CMV are also necessary, as the susceptibility of sweet potatoes to CMV has recently, also been found to be dependent upon their prior infection with the virus-like agent (J. Cohen, personal communication).

The aphid and whitefly catches at the four locations (Embu, Kakamega, Katumani and Muguga) showed that the known vector species of some of these viruses occur in abundance especially during the crop growing season. The aphid species (Table 20 and Figs 45-48) that are reported to transmit SPFMV and CMV, including *Myzus persicae, Aphis craccivora, Lipaphis erysimi* and *Aphis gossypii*, were especially abundant during the cropping season. The vectors of SPLV, SPCSV and SPC-LV are unknown, and it is now necessary to determine whether any of the known vectors of SPFMV and/or CMV are also able to transmit one or more of the viruses; it is also necessary to determine whether other aphid species that were trapped in large numbers such as *Aphis fabae, Aphis spiraecola, Brevicoryne brassicae, Rhopalosiphum maidis* and *Toxoptera citricidus* are vectors of the three viruses and also, perhaps, of SPFMV.

The whitefly, *Bemisia tabaci*, the vector of SPMMV (Hollings *et al.*, 1976) was also trapped in large numbers (Table 22); this explains the high incidence of SPMMV at all locations, and possibly that of other viruses for which the vectors are unknown such as SPLV, SPCSV, and SPC-LV. SPLV was especially prevalent in hot dry areas such as Rumuruti and Nanyuki, in the Rift Valley Province, and the hot humid Coast Province (Figs 5 and 9) where climate conditions allow whiteflies to thrive. This was further emphasised by whitefly catches (Figs 49-52) which showed that whitefly populations were lowest in the cold rainy cropping season from April to August, when aphids were most abundant. This further suggests that in such areas, whiteflies were active and effective virus vectors during the warm non-cropping season when

The apparent high incidence of SPLV in the re-infection experiments should be interpreted with caution as infection could have been present, but unrecognised, in the planting stock (see section 3.2.1).

The results of the re-infection studies at the four locations indicated that, during the first season, about 25% of the healthy plants became infected with SPFMV, SPMMV, and SPC-LV at Kakamega and about 15% with SPFMV at Embu; notably, however, those at the other two sites remained substantially free of infection. The results from Kakamega are especially important because this is the centre of the major sweet potato growing area in Kenya and, with such rapid re-infection, considerable difficulties would arise in the future development of appropriate strategies for disease control.

By contrast, re-infection during the first season was relatively low at Katumani and Muguga. This suggests that in these two areas farmers, if initially provided with elite virus-free stocks, could re-plant stocks for a second season with minimal virus-induced crop losses. However, as shown by the second assays, about 50% of stocks would probably be re-infected in all sites after the second season. Similarly, by the end of the 3rd season, 80-95% of the stocks would probably be re-infected.

The results of the studies on the possible relationship between virus spread and the proximity of healthy plants to the foci of infection indicated that distance (25-50 metres) was important in reducing infection (<10%), but only during the first cropping season. This suggests that, especially in Central Province where sweet potatoes are grown as a perennial crop, elite virus-free stocks would need to be planted at a greater distance from the infected stocks to minimise re-infection.

The direction of the prevailing wind had some, though not great, effect on the spread of the viruses, and needs to be considered when pathogen-free planting stocks are planted near older infected crops.

The regular insect catches showed that (Figs 37, 39, 41, 43) aphid populations increased to a major peak in June, and a minor peak in September/October. The first peak coincides with the period of long rains and the major cropping season in most parts of Kenya, while the minor peak coincides with the period of short rains during which less important short season crops are grown. This indicates that effective control of aphid borne viruses such as SPFMV would, together with other measures necessitate control of aphids to prevent the development of large populations.

The whitefly catches at all sites (Figs 49-52) showed that their population trends differed from those of aphids. Thus, the whiteflies reached a major population peak in February and a minor peak in September/October, the latter coinciding with the minor population peak for aphids. The whitefly catches were extremely low in April to July, when the aphids were especially abundant, and also during the main cropping season. This indicates that the cold and wet conditions during the main cropping season are unfavourable for whiteflies. It also indicates that most spread of whitefly-borne viruses such as SPMMV occurs early and or late in the season; effective control of whiteflies would, therefore, be necessary before the expected period of spread.

The aphid and whitefly catches were well correlated with the spread of SPFMV and SPMMV in the re-infection experiments. The spread of SPFMV was early and rapid in the second cropping season. That of SPMMV was slow and late in the second season, but comparable to that of SPFMV during the third season. This implies that the populations of whiteflies increased and re-infected the stocks during the non-cropping period when aphid populations were low.

CHAPTER 4

CHEMOTHERAPY AS AN ADJUNCT TO MERISTEM-TIP CULTURE FOR OBTAINING VIRUS-FREE SWEET POTATOES

4.1 INTRODUCTION

Meristem-tip culture (MTC) in these studies defined is as the culture of shoot apical meristematic dome plus one or two leaf primordia, measuring 0.5-0.1 mm in length. MTC was first used to obtain virus-free plants of dahlias (Morel and Martin, 1952). The method had wider applicability after the introduction of the basic Murashige and Skoog medium (1962), which has since been modified to facilitate the culture of different plant species. When combined with thermotherapy, MTC has been utilised to obtain virus-free plants of numerous vegetatively-propagated species (e.g. Quak, 1970; Frison, 1981; Mori, 1971; Vasil, 1980; Jarret *et al.*, 1986; Templeton *et al.*, 1985; Sharp *et al.*, 1984; Sood *et al.*, 1982; Walkey, 1980; Cooper, 1978; Walkey, 1976; Walkey & Freeman, 1977).

Thermotherapy, shoot-tip culture (STC) defined as the culture of shoot apical tips measuring 3-4 mm long), MTC, and regeneration procedures have been used successfully for obtaining virus-free sweet potato plants, for germplasm storage, propagation and international exchange of germplasm (e.g. Alconero *et al.*, 1975; Liao *et al.*, 1979; Litz *et al.*, 1978; Over de Linden *et al.*, 1971; Ng, 1987; Xin *et al.*, 1987; Love *et al.*, 1985; Kuo *et al.*, 1985; Scaramuzzi *et al.*, 1983; Hwang *et al.*, 1983; Sihachakr, 1982; Chen, 1981; Nome *et al.*, 1980; Templeton, 1986; Rey *et al.*, 1985; Frison, 1982; McCrae, 1982; Hwang *et al.*, 1981; Moyer, 1988).

Although thermotherapy and MTC have been used to obtain virus-free plants,

there are constraints to their wider application. The tiny shoot-tip (0.5 mm) that must be excised to achieve virus elimination from some species often results in poor survival of plants or, if successful prolonged growth periods (Wambugu *et al.*, 1985). Moyer (1988) recently reviewed the main problems of MTC of sweet potatoes which included contamination, mortality (25-40%), lack of regeneration of cultures, difficulties in defining media composition (especially hormone and sucrose contents), and dehydration during culture due to the type and concentration of agar in the medium. Although the technique has great potential for crop improvement, its application has been limited to relatively few scientific institutions and commercial laboratories because it is very expensive in terms of trained personnel and laboratory equipment. In developing countries, in which such techniques could provide virus-free germplasm for many crop improvement programmes and so increase food production, the technique is not widely used. In order to improve this situation, attempts have been made to develop alternative methods and to improve the efficiency of MTC.

The use of antiviral chemicals as additives to culture media has enormous potential value to agriculture, particularly for virus elimination in perennial crops that are propagated vegetatively and in which viruses can cause severe crop losses. Dawson (1984) evaluated the efficacy of 27 reagents known to be active against animal viruses, and found 14 of them to inhibit the multiplication of plant viruses. Recent studies have suggested that virus multiplication in plant callus cultures may be suppressed by the incorporation of the nucleoside analogue ribavirin into the culture medium (Cassells *et al.*, 1980; Cassells *et al.*, 1982; Simpkins *et al.*, 1981 and Shepard, 1977), or by the incorporation of a mixture of ribavirin and Vidarabine (Lozoya *et al.*, 1984). Incorporation of ribavirin in culture media has also been reported to be effective for eliminating virus from shoot-tip and meristem cultures

of a wide range of plant species (Schider 1983; Wambugu *et al.*, 1985; Cassels *et al.*, 1982; Hansen, 1979; Klein *et al.*, 1982; Sidwel *et al.*, 1972; Vasti, 1973; Griffiths *et al.*, 1990; Albouy *et al.*, 1988; and Deogratias *et al.*, 1989). However, ribavirin, especially at high concentrations, has been reported to cause growth retardation. Schuster (1977), however, indicated that abscisic acid as a medium additive compensated for the damage caused, and prevented the deleterious effects. Reports by Klein (1982) and Wambugu (1985) indicated that, when large shoot-tip cultures (3-4 mm) were grown in the presence of Ribavirin (10 mg/l), high levels of virus eradication (80%) could be achieved with negligible growth inhibition, and within a reasonably short period of plantlet regeneration (4-6 months). The molecular basis of chemotherapy and the mechanism of action of Ribavirin has been well described (Simon *et al.*, 1973; Streeter *et al.*, 1973). These authors indicate that Ribavirin is an analogue for guanine and inhibits viral replication by impairing RNA synthesis. As Ribavirin does not discriminate between host and viral RNA, it can retard the growth of the host.

Recent reports have also indicated that DHT (2,4-dioxo hexahydro, 1,3,5-triazine) is effective when added to culture media in inhibiting viral replication without having phytotoxic effects (Borisenko *et al.*, 1985; Bittner *et al.*, 1986; Bogusch *et al.*, 1985; Schuster, 1987). Bogusch *et al.* (1985) reported that when 1 ml of 0.01% DHT was injected into axillary buds of cherry infected with prunus necrotic ringspot virus, 65% of plants grown from the cultured buds were virus-free. DHT has also been investigated as a virus inhibitor when applied as field sprays. Schuster *et al.* (1981) reported that 4-5 sprays of 80% DHT reduced the effects of virus infection in field grown potatoes and increased yields by 6.5% in a field that was 24.6% infected. Schuster (1982) reported that a mixture of 100 mg/l DHT and 50 mg/l Ribavirin, when applied in field sprays, reduced the deleterious effects in

untreated leaves and resulted in increased crop yields. Byhan *et al.* (1986) reported that DHT and M-phenyl-N-p-carboxyphenyl-thiourea, (1% in mixtures) had synergistic effects and, when sprayed on tobacco plants, reduced the concentration of tobacco mosaic virus (TMV) in leaves to a level at which the virus was not serologically detectable. By use of a radioactive carbon marker, thiourea was shown to prevent the breakdown of DHT, and so maintain its concentration effect. The mode of action of DHT on host and viral RNA synthesis and on cytoplasmic membranes has been studied using PVX in tobacco as a model system (Schuster *et al.*, 1981; 1986; 1984). Such studies have revealed that DHT, like Ribavirin, acts as an analogue for the pyrimidine base uracil, and inhibits virus replication by inhibiting viral RNA synthesis.

Other reports indicate that 2-thiouracil (2 mg/l) effectively eradicated virus from 46.6% of infected potatoes (Borissenko *et al.*, 1985). Cyanoguanidine was also reported by Borissenko (1985) to effect 28.6% viral eradication in potato. Horst (1974) reported that amantadine supplements in tissue culture medium helped to free chrysanthemums from chrysanthemum stunt viroid. Stone (1982) reported the successful eradication of viruses from *Ullucus tuberosus* by MTC and chemotherapy using Vidarabine (1 mg/l) and suggested that Vidarabine promoted growth.

In a search for novel chemotherapeutants, Schuster *et al.* (1987) showed that application of 0.5 mM 5-fluororotic acid (5FOA) completely inhibited the replication of PVX in tobacco plants without any phytotoxic effects. He described 5FOA as a potent selective viral inhibitor.

Attempts to improve the efficacy of MTC (tips 0.5-1.0 mm long) or STC (3-4 mm long) for obtaining virus-free sweet potato plants by incorporating chemotherapeutants in culture media are now described and discussed.

4.2 MATERIALS AND METHODS

4.2.1 Sweet Potato Genotypes, Maintenance and Culture Methods

The sweet potato genotypes used were H1B 687, Papota, 157 and Jasper; their origin, subsequent maintenance and virus contents were as described previously (2.1 and 2.2).

Stem pieces with several nodes from which the leaves had been removed were cut into smaller pieces, each with two lateral buds. These smaller pieces were surface sterilized by dipping them for 3 min in 70% ethanol, and then for 10 minutes in 1.5% sodium hypochlorite containing a few drops of Tween 20 (BDH Co. Ltd.). They were finally thoroughly washed four times in distilled sterile water.

Meristem-tips (0.5-1.0 mm) or shoot-tips (3-4 mm) were surgically excised in a laminar flow cabinet and cultured on MS-basal medium (Table 6) supplemented as for stages I & II (Table 7) for either meristem-tip or shoot-tip culture, to which had been added the appropriate test chemotherapeutant, as described thereafter (4.2.2).

4.2.2 Chemotherapeutants

1. 2-thiouracil (2-Thio) (4-Hydroxy-2-thiopyrimidine) mol. wt 128.1 from Sigma Co. Ltd dissolved in 1 NaOH, and filter-sterilized before use.
2. 2, 4-Dioxohexahydro- 1, 3, 5 -Triazine (DHT), mol. wt 115.0 from Aldrich Chemical Co. Ltd USA, dissolved in 2 N NaOH, and filter-sterilized before use.
3. 1-β-D-Ribofuranosyl-1, 2, 4 - triazole 3-carboxamide (ribavirin also called Virazole) (RB) mol. wt 224.2, obtained from Sigma Co. Ltd and Serva Feinbiochemica Heidelberg, New York, USA, dissolved in water, and filter-sterilized before use.

4. Adenine 9-β-D- arabinofuranoside (Vidarabine or Vira-A) (VID) mol. wt 267 obtained from Sigma Co. Ltd, dissolved in 1 N NaOH and autoclaved with the media.

4.2.3 Meristem-tip Cultures (MTC)

For MTC, the test chemotherapeutants included Ribavirin (RB) at concentrations of 0, 10, 20, 40 and 60 mg/l; DHT at 0, 10, 20, 40, 60, 80 and 100 mg/l; Vidarabine (V1D) at 0, 2, 4, 6 and 8 mg/l; 2-thiouracil (2-Thio) at 0, 5, 10, 15 and 20 mg/l, and the following combinations of anti-viral compounds:- RB 15 + VID 40 mg/l, RB 10 + DHT 100 mg/l, 2-Thio 5 + DHT 100 mg/l, RB 50 + DHT 50 mg/l, DHT 50 + RB 100 and DHT 100 + RB 50. Sweet potato genotypes H1B 687 and Papota were used in these studies except those with VID for which 157 was used. The treatments were replicated 24 times, and were for 4 weeks.

4.2.4 Shoot-tip Cultures (STC)

For STC, DHT was tested at concentrations of 0, 30, 60, 90, 120 and 150 mg/l with genotypes 560, Papota, Rose Centennial and T1B 9; each test was replicated 48 times.

4.2.5 Media Preparation and Culture Incubation

Media were prepared as described previously (2.3) and chemotherapeutants (4.2.2) were either added before or after autoclaving, and then dispensed in to the appropriate culture vessels.

The MTC or STC were incubated in the growth room under the conditions described (section 2.4) until they had regenerated into plantlets. The number of internodes, length and fresh weight of 24 plantlets per treatment were then recorded

before the plants were acclimatized (5.2.3) and transferred to a greenhouse. A summary of the type of culture, medium, culture vessels and time intervals for each stage of growth for plantlet regeneration are given in Table 24.

4.2.6 Virus Testing

The regenerated plantlets in the greenhouse were tested for virus infection by either DAS-ELISA (2.5.4) or NCM-ELISA (2.5.5).

4.2.7 Field Experiments (in Kenya)

Some of the regenerated plants from MTC, RB and DHT treatments that tested virus-free were further tested in field experiments in Kenya to investigate whether the chemicals had long term deleterious effects on the growth and yield of foliage and tubers. The experiment was planted with 12 sweet potato cuttings (each 20 cm long) from greenhouse plants of genotypes H1B 687 and Papota, from each chemical treatment. The cuttings were planted 75 cm apart in rows 50 cm apart, as in normal cultural practice, in a well-prepared and isolated flat plot. The experimental plot was not irrigated and was maintained for 6 months, the usual cropping season in Kenya. Plants were regularly observed for abnormalities. The weight of the foliage and tubers for each of the 12 plants in the row were measured using a spring balance and recorded separately for each treatment.

Table 24 STAGES OF PLANTLET REGENERATION FROM MERISTEM-TIP (MTC) OR SHOOT-TIP CULTURES (STC)

Stage	Type of culture	Media MS (Table 6)+ Supplements (Table 7)	Culture vessel	Place of incubation	Time of incubation (wk)
1	MTC (0.5-1.0 mm)	MS + Stage I	5 cm deep Petri dish	Growth room	8
2	STC (3-4 mm)	MS + Stage II	5 cm deep Petri dish	Growth room	4
3	Plantlets	MS + Stage II	100 ml specimen bottles	Growth room	4
4	Plantlets	MS + Stage III	150 ml specimen bottles	Growth room	4
5	Plantlets	Fisons C2 compost	5 inch pots or seedbox	Greenhouse	8

Chemotherapy treatment only in stages 1 & 2.

MTC - 24 replicates

STC - 48 replicates

Incubation conditions 25∓1°C, 16 hr photoperiod

30 $\mu Mm^{-2}S^{-1}PAR$

Plantlet regeneration time - STC 4 months, MTC 7 months

4.2.8 Statistical Analysis of Data

A 2-way analysis of variance (cross/nested) was carried out to test the significance the two factors (GENOTYPE and TREATMENT) have on plant growth by considering the changes in the dependent variables (NODES, LENGTH and FRESH WEIGHT), and taking into account the various effects (Vidarabine, Ribavirin and DHT).

Due to the unbalanced nature of the data, a General Linear Model (GLM) using subroutines from the statistical package SAS was used to carry out the significance test at 5% level. Normalization of the data was achieved by transforming the dependent variables as follows:

TNODES = SQRT (NODES)	square root
TLENGTH = LOG 10 (LENGTH)	log to base 10
TFRWGHT = LOG 10 (FRWEIGHT)	log to base 10.

Means and standard errors were calculated from the untransformed data.

The mean comparison test was performed at 5% level using the Duncan Multiple Range Test. Test for orthogonal polynomial contrasts were carried out to determine the trend (LINEAR, QUADRATIC, etc) of the FACTORS on the plant growth (Spector et al., 1987).

4.3 RESULTS

4.3.1 The Effects of Different Chemotherapeutants on Meristem-tip and Shoot-tip Cultures

As will be shown in the data for all three growth parameters (numbers of internodes, stem length and total fresh weight), all the chemotherapeutants tested had significant effects on the growth of sweet potato plantlets derived from meristem-tip (MTC) and shoot-tip cultures (STC).

All the compounds had similar effects on MTC-derived plantlets, although the effects of DHT in STC-derived plantlets were significantly different. DHT substantially decreased the growth of plantlets; Ribavirin and combinations of chemotherapeutants also had growth inhibiting effects, whereas growth retardation was least with Vidarabine.

DHT was the most effective anti-viral additive; although Vidarabine at appropriate concentrations was also moderately effective, it had less deleterious effect on the growth of plantlets.

Although the genotypes tested reacted similarly to the individual chemotherapeutants, there was some differential responses to the use of compound mixtures.

The effects of each of the compounds on growth of plantlets *in vitro*, virus elimination, plant survival and yield of MTC-derived plants grown in the field are now recorded and discussed.

4.3.2 Effects of 2-thiouracil on MTC

This compound, at all concentrations tested, was phytotoxic to sweet potato *in vitro* cultures. As no plantlets were regenerated from any of the cultures, the compound was not investigated further.

4.3.3 Effects of Vidarabine on MTC

The effects of Vidarabine at a range of concentrations (0-8 mg/l) on the growth of plantlets and the elimination of virus therefrom are summarized in Table 25 and illustrated in Figures 53 and 54a,b,c.

At a concentration of 4 mg/l, Vidarabine stimulated the growth of plantlets to a remarkable and highly significant degree; at higher concentrations it significantly inhibited growth, whereas at 2 mg/l it had only a slight stimulatory effect. Vidarabine at all concentrations had comparable effects on the number of internodes, stem length and fresh weight of plantlets.

Virus assays of plantlets showed that Vidarabine had only a slight beneficial effect on virus elimination, especially at a concentration of 4 mg/l. Thus, although the compound stimulated plantlet growth, its use as an antiviral additive to cultures media has very limited potential for sweet potato.

Table 25. EFFECTS OF VIDARABINE CONCENTRATIONS ON VIRUS ELIMINATION AND GROWTH *IN VITRO* OF SWEET POTATO PLANTLETS DERIVED FROM MTC

Conc. (mg l^{-1})	Virus elimination * (%)	Internodes** (No.)	Length (mm)	Fresh wt (g)
0	27	6^{b}	25^{b}	0.10^{b}
2	31	6^{a-b}	29^{a-b}	0.12^{b-c}
4	39	8^{a}	45^{a}	0.22^{a}
6	35	5^{c}	18^{c}	0.07^{c-c}
8	33	4^{d}	8^{d}	0.03^{d}

Means with the same letter are not significantly different at P=0.05 (Duncan's Multiple Range Test).

* Mean of 17 plants per treatment of genotypes H1B 687 and 157.

** Mean of 24 plants per treatment of the two genotypes.

Basal medium: MS supplemented 3% (W/V) sucrose.

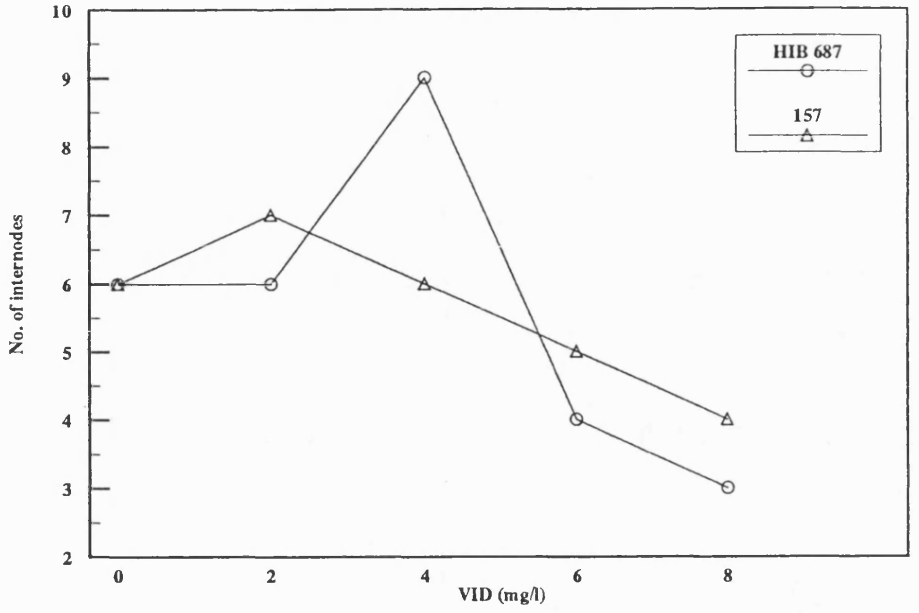
Incubation conditions: $25 \mp 1°C$, 16 hr photoperiod

($30\ \mu Mm^{-2}S^{-1}PAR$).

Fig. 53

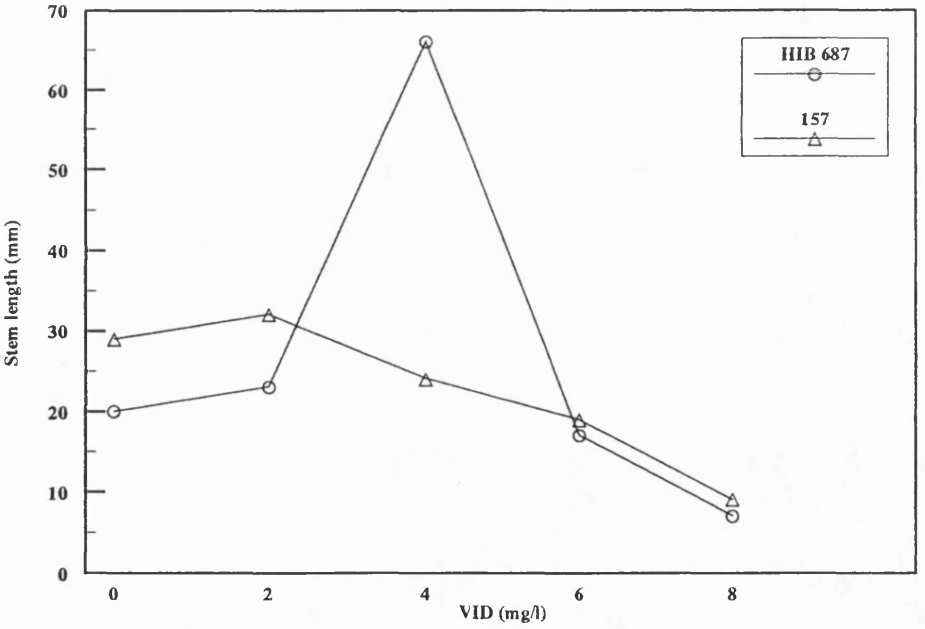
Effects of vidarabine on plant growth; sweet potato genotype 560 (HIB 687).

Fig. 54a. EFFECTS OF VIDARABINE ON No. OF INTERNODES (meristem-tip culture)*



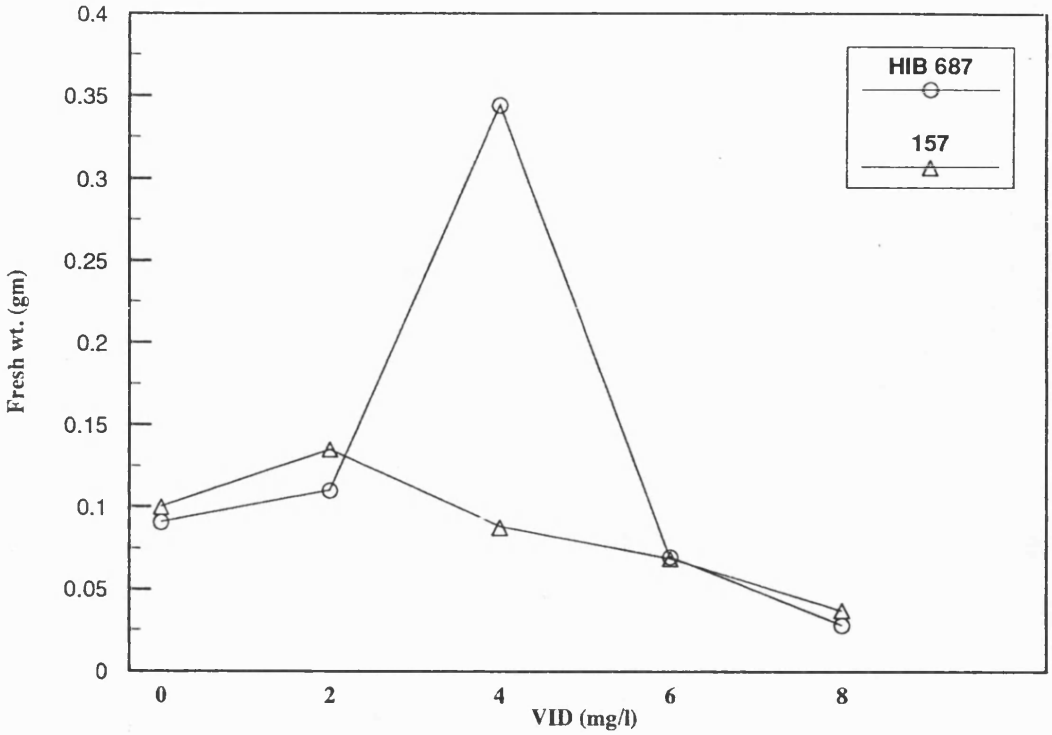
* Mean of 12 plants per treatment

Fig. 54b. EFFECTS OF VIDARABINE ON PLANT LENGTH (meristem-tip culture)*



* Mean of 12 plants per treatment

Fig. 54c. EFFECTS OF VIDARABINE ON FRESH WEIGHT (meristem-tip culture)



* Mean of 12 plants per treatment

4.3.4 Effects of Combined Chemotherapeutants on MTC

The effects of combined chemotherapeutants on the growth of MTC are summarised in Table 26 and illustrated in Figure 55a,b,c; it was possible to assay plants for virus infection from only one treatment (Rb 10 + DHT 100).

Statistical analysis showed that the RB 15 + VID 40 treatment had a significantly greater effect on growth (internode number and shoot length) than other treatments, and that three treatments (RB 50 + DHT 100, DHT 50 + RB 100 and DHT 100 + RB 50) had a significantly greater inhibitory effect. Both genotypes tested (H1B 687 and Papota) obviously tolerated RB 10 + DHT 100 and 2-Thio 5 + DHT 100 better than other compound combinations as shown in Figure 55a,b,c; only very stunted plants were obtained from cultures tested with DHT 50 + RB 100.

Of the cultures grown in media supplemented with RB 15 + VID 40, only those of the genotype H1B 687 produced plantlets; conversely, only plantlets of cv. Papota were regenerated from cultures treated with RB 50 + DHT. Although further tests of this phenomena are necessary, these results indicate that genotypes respond differently to chemotherapeutants when alone or in mixtures.

Virus assays indicated that concentrations of RB of 10 mg/l and DHT of 100 mg/l were especially effective in facilitating virus elimination from MTC-derived plantlets. It was unfortunate, therefore, that it was not possible to assay plantlets from other treatments.

Table 26. EFFECTS OF COMBINED CHEMOTHERAPEUTANTS ON VIRUS ELIMINATION AND GROWTH OF cv. PAPOTA AND GENOTYPE H1B 687

		Effect on growth *in vitro* **		
chemotherapeutants conc. (mg/l)	Virus elimination (%)*	Internodes (No.)	Length (mm)	Fresh wt (g)
RB (15)+VID (40)	-	6^{b}	30^{c}	0.10^{c}
RB (10)+DHT (100)	71	5^{c}	20^{d}	0.11^{c}
Thio (5)+DHT (100)	-	5^{c}	20^{d}	0.07^{c}
RB (50)+DHT (50)	-	3^{d}	9^{e}	0.04^{d}
DHT (50)+RB (100)	-	3^{d}	10^{e-f}	0.04^{d}
DHT (100)+RB (50)	-	2^{d}	7^{f}	0.03^{d}

Means with the same letter are not significantly different at P=0.05 (Duncan's Multiple Range Test)

* Mean of 24 plants of cv. H1B 687 per treatment

** Mean of 24 plants per treatment

Basal medium: MS supplemented 5% (w/v) sucrose

Incubation conditions: $25 \mp 1°C$, 16 hours photoperiod

$(30\ \mu Mm^{-2}S^{-1}PAR)$

Fig. 55a. EFFECTS OF COMBINED CHEMOTHERAPEUTANTS ON THE No. OF INTERNODES (Meristem-tip culture)*

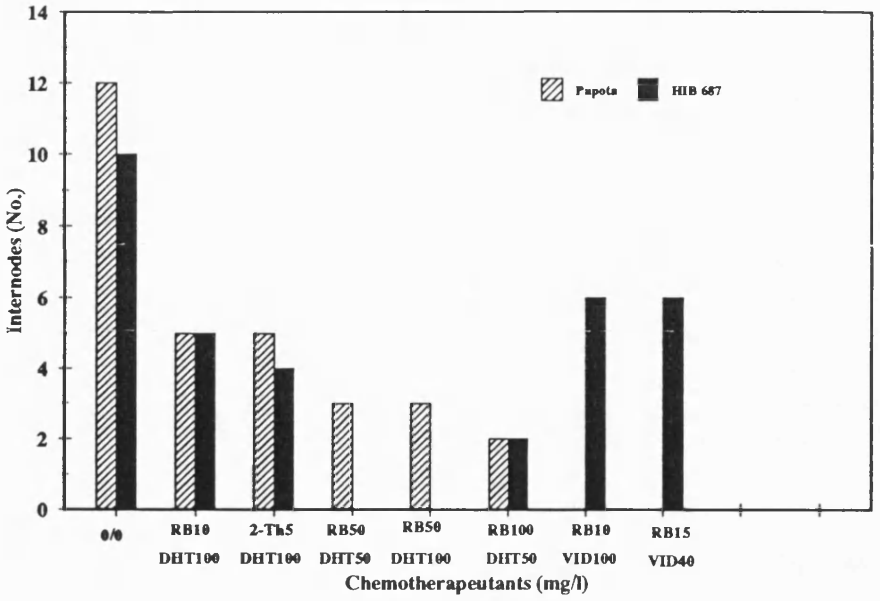
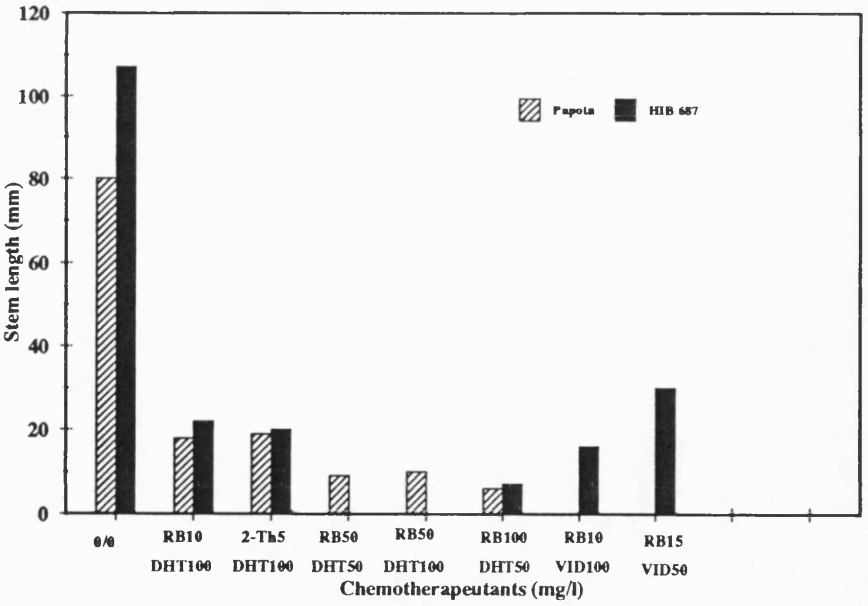
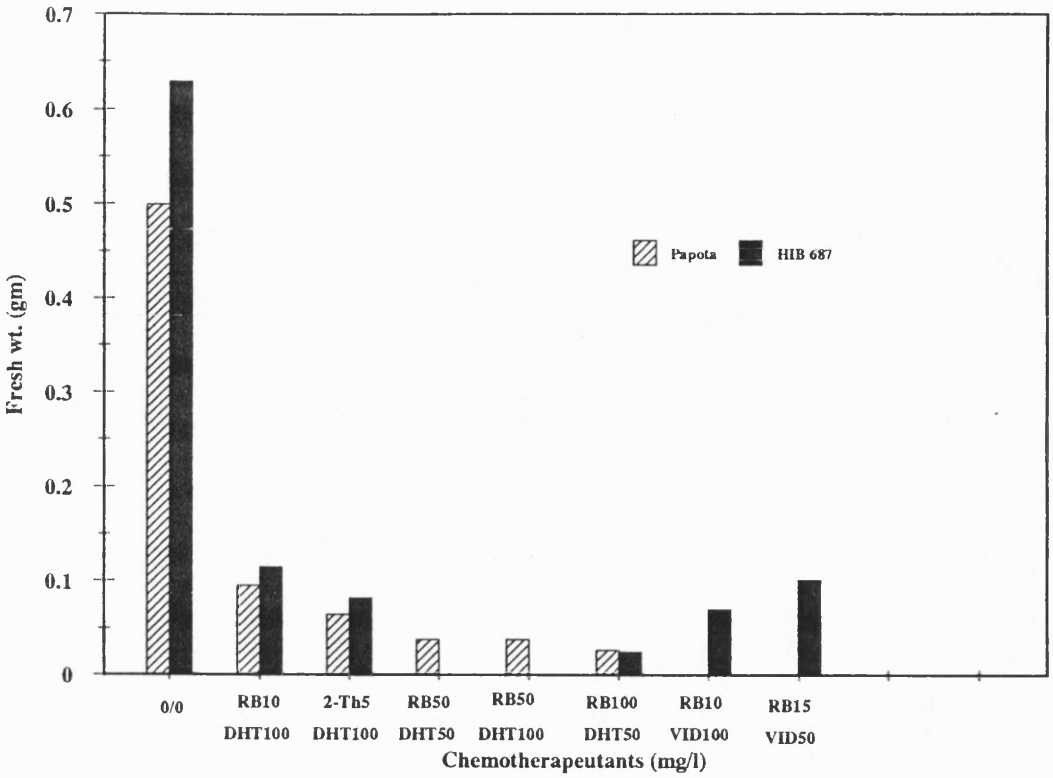


Fig. 55b. EFFECTS OF COMBINED CHEMOTHERAPEUTANTS ON THE PLANT LENGTH (Meristem-tip culture)*



* Mean of 12 plants per treatment

Fig. 55c. EFFECTS OF COMBINED CHEMOTHERAPEUTANTS ON FRESH WEIGHT (Meristem-tip culture)*



* Mean of 12 plants per treatment

4.3.5 Effects of Ribavirin on MTC and Field-grown MTC-derived plants

The growth of MTC, and their field-grown derivatives, was decreased inversely with increasing concentrations of Ribavirin used in the culture media. However, the success of virus elimination increased with increasing Ribavirin concentration, although there was a concomitant decrease in survival at Ribavirin concentrations exceeding 10-20 mg/l. The effects of Ribavirin on the growth of MTC-derived plantlets, virus elimination, survival of plantlets and the subsequent growth of field-grown plants are summarised in Table 27 and illustrated in Figure 56a,b,c.

The illustrations clearly show that, although internode number was similar for plantlets of both genotypes, the growth (as measured by stem length and fresh weight) of H1B 687 exceeded that of cv. Papota. Moreover, the results show that Ribavirin had a significant growth inhibitory effect on plantlets, with deleterious effects increasing concomitantly with increasing concentration of compound added to the media.

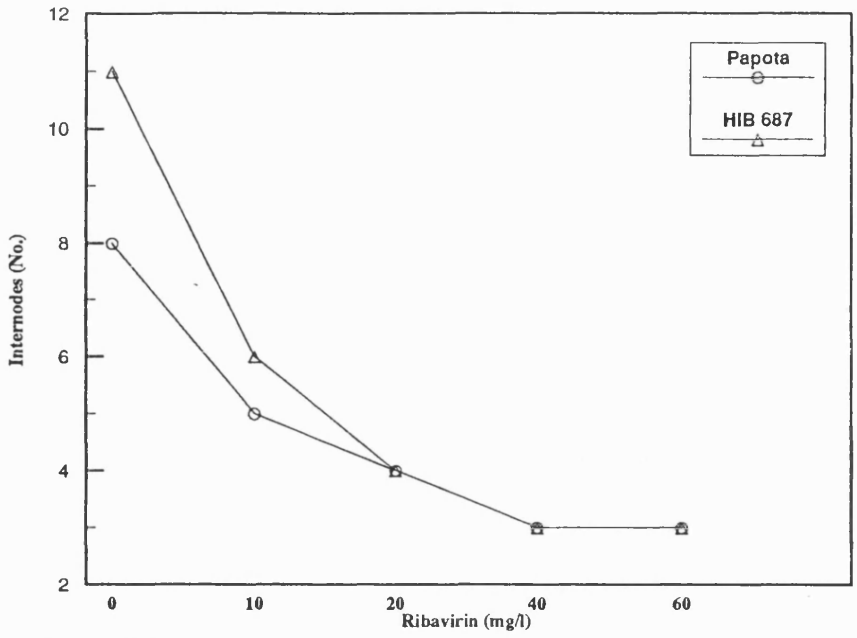
An assessment of the performance of field-grown plants originating from treated MTC indicated that there was a "carry-over" effect of Ribavirin on the growth of the plants. Thus, the average weight of foliage and tubers of plants regenerated from MTC to which no Ribavirin was added substantially exceeded that of plants derived from MTC grown in media containing 10-60 mg RB/l; greater deleterious effects generally occurred in plants originating from MTC grown in media containing 20 mg or more RB/l.

Table 27 EFFECTS OF RIBAVIRIN ON GROWTH (IN VITRO AND THE FIELD) AND VIRUS ELIMINATION OF cv. PAPOTA AND GENOTYPE H1B 687

			Effect on growth**				
			In vitro			In field***	
RB conc. (mg/l)	Survival ** (%)	Virus elimination (%)*	Internodes (No.)	Shoot Length (mm)	Fresh wt (g)	Foliage (g)	Tubers (g)
0	73	32	10^{b-c}	44^{b-a-c}	0.22	688	983
10	70	50	6^{e-d-c}	$28^{b-e-d-c}$	0.11^{b-c-d}	546	588
20	63	59	5^{e-d-c}	$20^{f-e-d-c}$	0.07^{b-c-d}	375	496
40	55	67	3^{e-f}	11^{f-e-g}	0.04^{e-c-d}	591	500
60	49	62	3^{e-d-f}	8^{f-g}	0.04^{e-d}	423	345

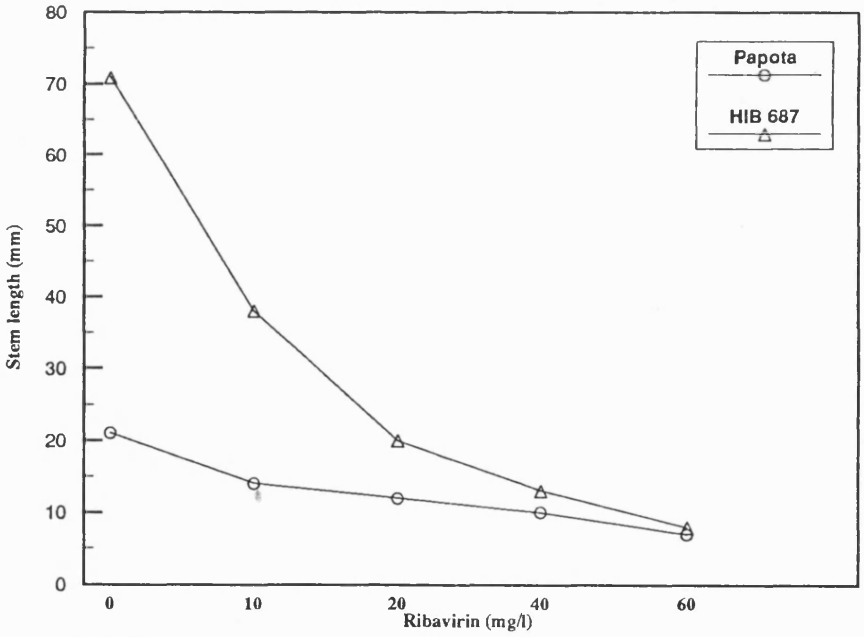
Means with the same letter are not significantly different at P=0.05 (Duncan's Multiple Range Test).
* Mean of 16 plants per treatment.
** Mean of 24 plants of cv. Papota and genotype H1B 687 per treatment.
*** Mean of 36 plants of cvs Papota, Rose Centennial, and genotype H1B 687.
Average survival = 62%.
Average virus freedom = 60%.
Basal medium: MS supplemented 5% (w/v) sucrose.
Incubation conditions: 25°C, 16 hr photoperiod
(30 $\mu Mm^{-2}S^{-1}PAR$).

Fig. 56a. EFFECTS OF RIBAVIRIN ON No. OF INTERNODES (meristem-tip culture)*



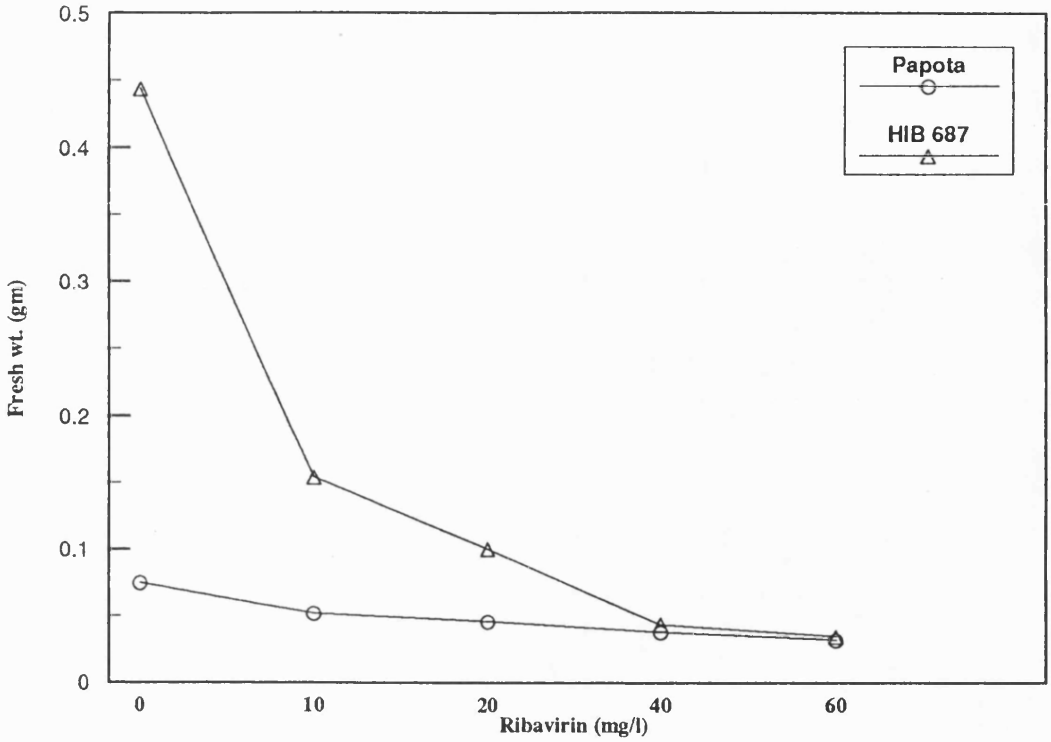
* Mean of 12 plants per treatment

Fig. 56b. EFFECTS OF RIBAVIRIN ON PLANT LENGTH (meristem-tip culture)*



* Mean of 12 plants per treatment

Fig. 56c. EFFECTS OF RIBAVIRIN ON FRESH WEIGHT (meristem-tip culture)*



* Mean of 12 plants per treatment

4.3.6 Effects of DHT on MTC

Like Ribavirin, DHT caused a decrease in the growth of MTC and their field-grown derivatives; the extent of the effect increased with increasing compound concentration, although to a lesser extent than Ribavirin. Plantlets in media containing DHT generally had a lighter green colouration *in vitro*, but developed a normal colour when subsequently grown in compost under greenhouse conditions. The effects of DHT on the growth of MTC-derived plantlets, virus elimination, survival of plantlets and the subsequent growth of field-grown plants are summarised in Table 28 and illustrated in Figure 57a,b,c.

The results show that, at a concentration of 20 mg/l, DHT had no significant effect on internode number, but at higher concentrations decreased internode development appreciably. Shoot length and fresh weight were significantly decreased at all concentrations of DHT tested, the deleterious effect increasing with increasing concentrations of compound added to the media.

The growth of field-grown plants derived from treated MTC indicated that, like Ribavirin, DHT had a "carry-over" effect. Thus, the growth of plants grown from DHT-treated MTC was decreased substantially, the effect being most obvious in the greatly reduced yields of tubers; like Ribavirin, DHT-induced yield decreases increased inversely with compound concentration.

Virus assays indicated that DHT was effective in eliminating virus from MTC (61% overall); this beneficial effect of DHT was apparent in MTC plantlets obtained in media containing 20 mg DHT/l, but increased with increasing concentration of compound used (Table 28). Although the growth of plantlets *in vitro* was adversely affected by DHT, a high proportion (mean 70%) survived.

Table 28. EFFECTS OF DHT ON GROWTH (*in vitro* and the field) AND VIRUS ELIMINATION FROM cvs PAPOTA AND H1B 687

			Effect on growth **				
			In vitro			In field ***	
DHT conc (mg/l)	Survival ** (%)	Virus elimination (%)*	Internodes (No.)	Shoot Length (mm)	Fresh wt (g)	Foliage (g)	Tubers (g)
0	75	29	11^{a}	53^{a}	0.29^{a}	688	1129
20	71	54	9^{a}	25^{b}	0.14^{b}	-	-
40	70	59	6^{b}	20^{b-c}	0.09^{c}	604	479
60	69	65	5^{b-c}	14^{d-c}	0.06^{c}	675	750
80	65	60	4^{d-c}	12^{d}	0.05^{d}	467	417
100	63	67	3^{d}	10^{d}	0.03^{d}	407	312

Means with the same letter are not significantly different at P=0.05 (Duncan's Multiple Range Test).

* Mean of 22 plants of cvs Papota and H1B 687 per treatment.

** Mean of 24 plants of cvs Papota and H1B 687 per treatment.

*** Mean of 36 plants of cvs Papota, H1B 687 and Rose Centennial.

Average survival = 70%.

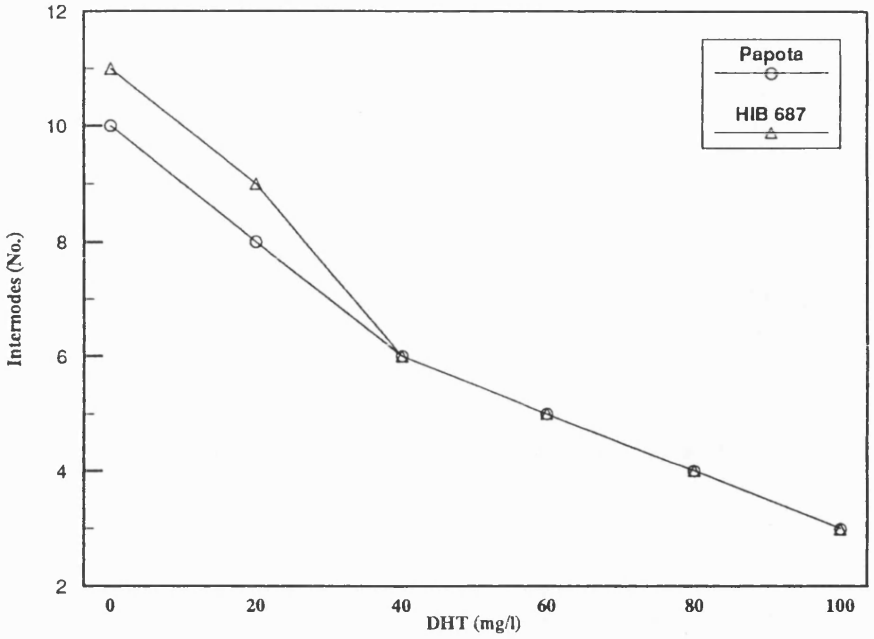
Average virus freedom = 61%.

Basal medium: MS supplemented 5% (w/v) sucrose.

Incubation conditions: 25°*C*, 16 hours photoperiod

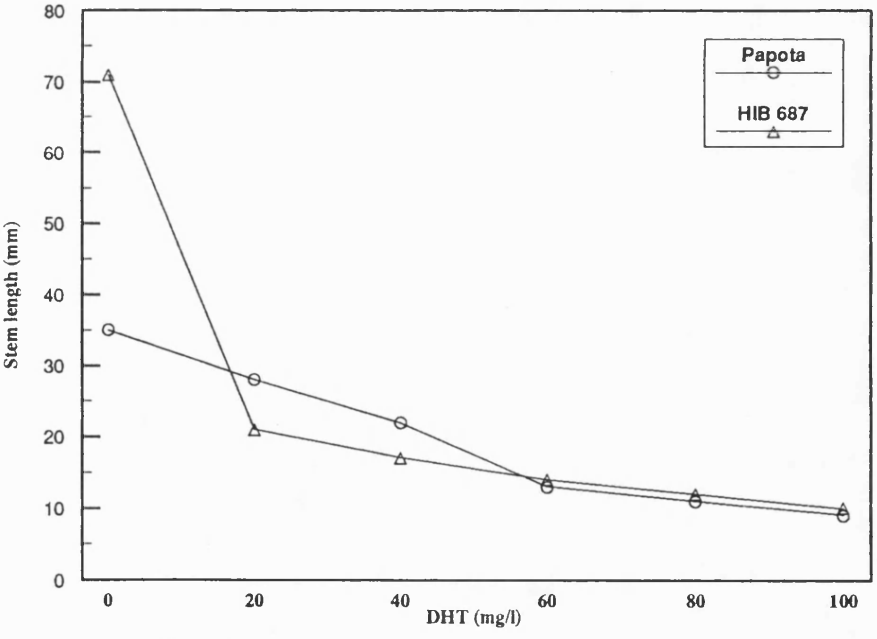
(30 $\mu Mm^{-2}S^{-1}PAR$).

Fig. 57a. EFFECTS OF DHT ON THE No. OF INTERNODES (meristem-tip culture)*



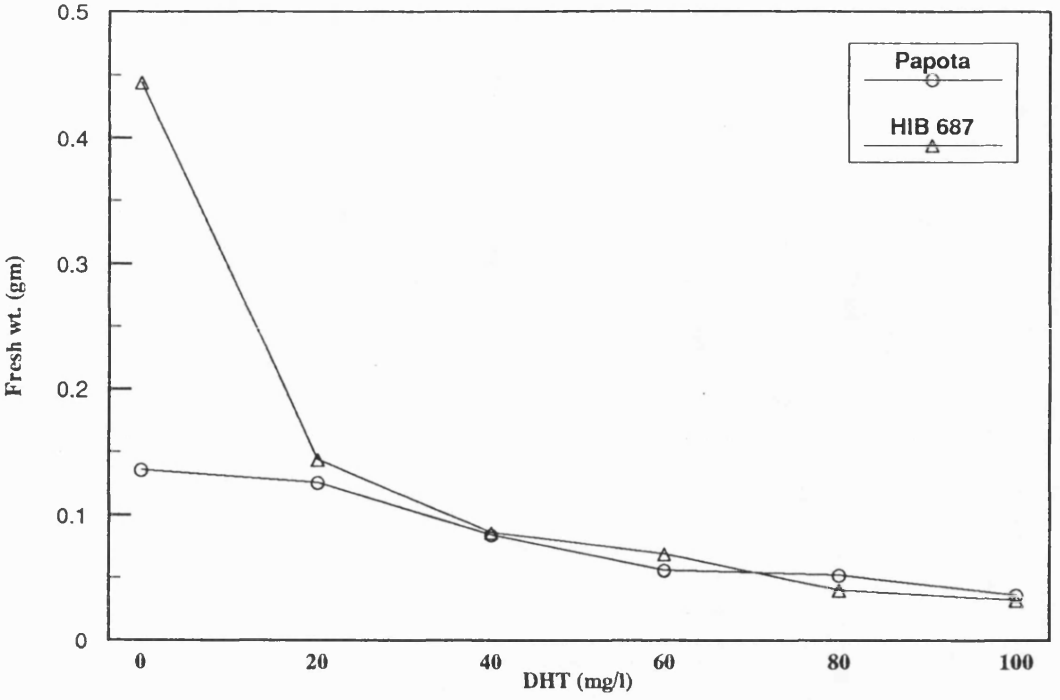
* Mean of 12 plants per treatment

Fig. 57b. EFFECTS OF DHT ON PLANT LENGTH (meristem-tip culture)*



* Mean of 12 plants per treatment

Fig. 57c. EFFECTS OF DHT ON FRESH WEIGHT (meristem-tip culture)*



* Mean of 12 plants per treatment

4.3.7 Effects of DHT on Shoot-tip culture

Most of the effects of DHT on STC were similar to those on MTC except for virus elimination and plant survival. The effects of DHT on the growth, virus elimination and plantlet survival of STC are summarised in Table 29 and Figure 58a,b,c & 59a,b,c. Statistical analysis of the data showed that the number of internodes was significantly reduced at all DHT concentrations, with the extent of the decrease increasing inversely with DHT concentration.

Similarly, although a DHT concentration of 30 mg/l caused a slight reduction in length and fresh weight of plantlets, higher concentrations had an inverse increasing significant effect on growth. Sweet potato STC however, were very otherwise tolerant of DHT; thus plantlets, although smaller (figure 56), mostly (average 95%) survived chemotherapy. Virus assays, however indicated that DHT was ineffective in eliminating viruses from STC (mean of all treatments = 8%); this contrasts with its effectiveness in facilitating the elimination of viruses (mean 61%) from MTC.

Table 29. EFFECTS OF DHT ON GROWTH AND VIRUS ELIMINATION OF SHOOT TIP CULTURES (STC)

	Effect on*				
DHT conc. (mg/l)	Survival (%)	Virus elimination (%)*	Internodes (No.)	Shoot Length (mm)	Fresh wt (g)
0	97	1	15^{a}	13^{a}	0.92^{a}
30	97	6	12^{b}	11^{a}	0.70^{a-b}
60	92	6	9^{c}	9^{b}	0.46^{b}
90	96	9	8^{c}	8^{b-c}	0.36^{c-b}
120	94	9	7^{d}	6^{d-c}	0.29^{c}
150	93	9	6^{e}	5^{d}	0.23^{d}

Means with the same letter are not significantly different at P=0.05 (Duncan's Multiple Range Test).

* Mean of 48 plants per treatment of cvs Papota, Rose Centennial and genotypes H1B 687 and Tib 9.

Average survival = 94%.

Average virus freedom = 8%.

Basal medium: MS supplemented 5% (w/v) sucrose.

Incubation conditions: 25°C, 16 hr photoperiod

(30 $\mu Mm^{-2}S^{-1}PAR$).

Fig. 58. Effects of DHT on plant growth (shoot-tip culture) of cvs Papota and 560 (HIB 687).

a. Effects of DHT on cv. HIB 687 14 week old shoot-tip cultures.

b. Effects of DHT on cv. HIB 687 18 week old shoot-tip cultures.

c. Effects of DHT on cv. Papota 14 weeks old shoot-tip cultures.

Fig. 58

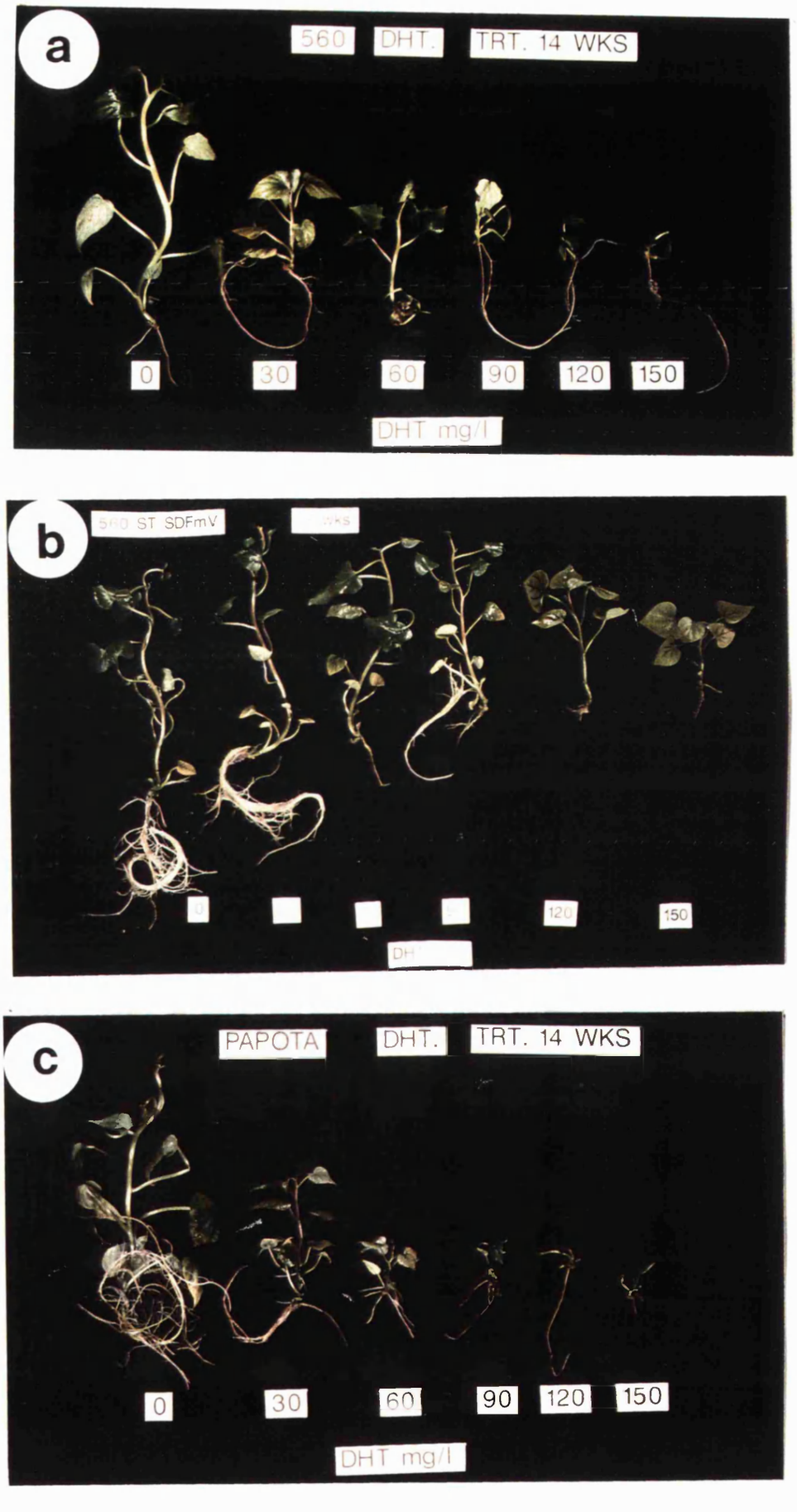

a
560
DHT.
TRT. 14 WKS
0
30
60
90
120
150
DHT mg/l
b
560 ST SDFmV
WKS
120
150
c
PAPOTA
DHT.
TRT. 14 WKS
0
30
60
90
120
150
DHT mg/l

Fig. 59a. EFFECTS OF DHT ON THE No. OF INTERNODES (Shoot-tip culture)*

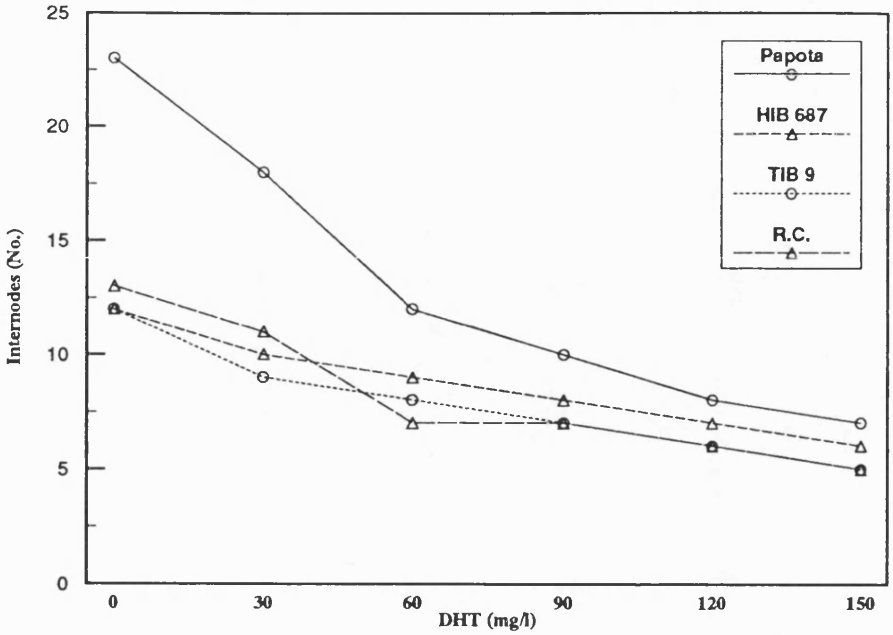
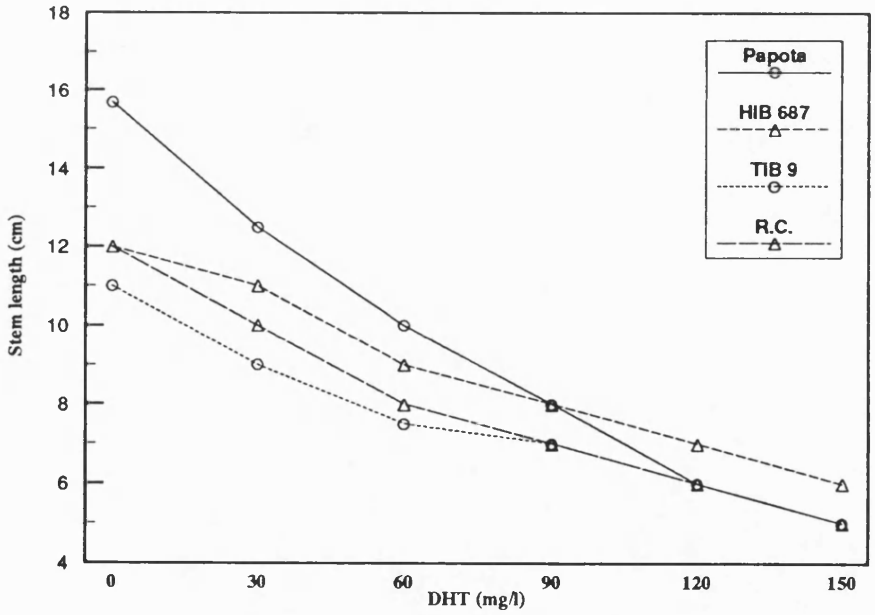


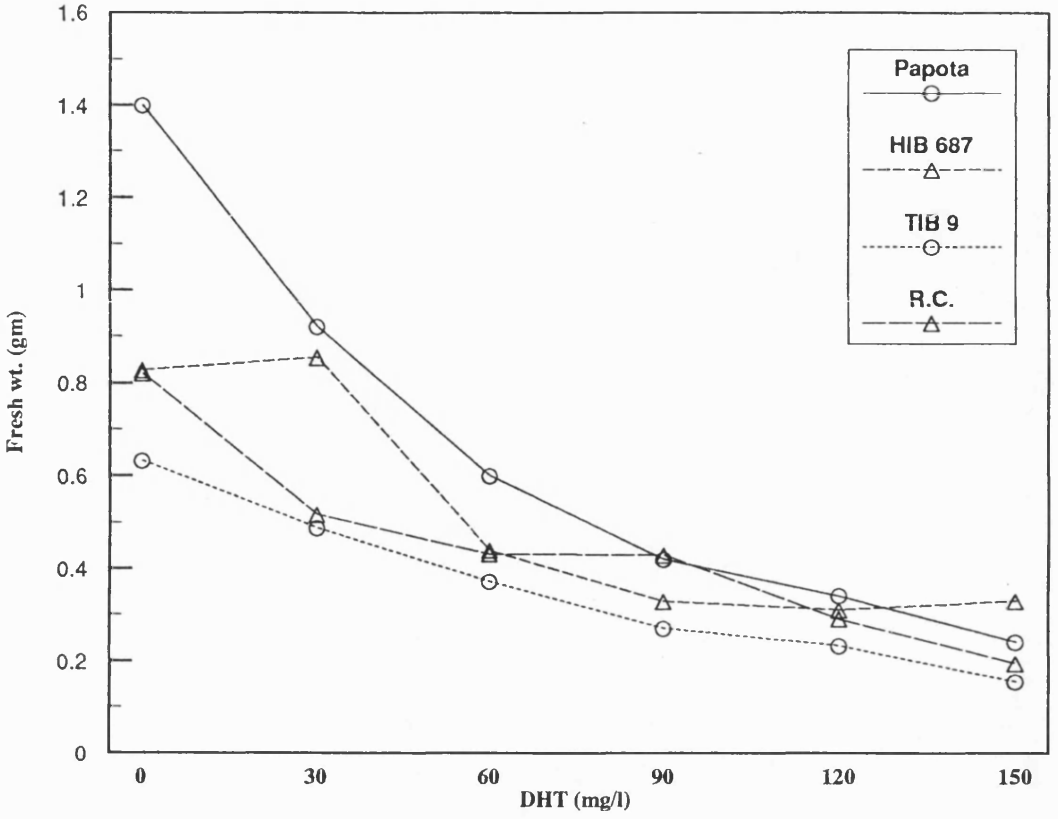
Fig. 59b. EFFECTS OF DHT ON PLANT LENGTH (Shoot-tip culture)*



* Mean of 12 plants per treatment

R.C.= Rose Centennial

Fig. 59c. EFFECTS OF DHT ON FRESH WEIGHT
(Shoot-tip culture)*



*** Mean of 12 plants per treatment**

R.C.= Rose Centennial

4.4 DISCUSSION

4.4.1 Aims of these studies

There were no previous reports on the use of antiviral chemicals for the elimination of sweet potato viruses from plantlets grown *in vitro*; it was hoped, therefore, that these studies might make an important contribution to the production of virus-free plants of this very important tropical tuber crop. Due to the lack of such information for sweet potato viruses, experiments were designed to explore the effects on different sweet potato genotypes of several chemotherapeutants reported to be effective with other virus-host combinations, either alone at several concentrations or in combination. The aim was to select one or two chemotherapeutants that might be used to significantly improve the existing MTC techniques for eliminations of viruses, but having minimal phytotoxicity. Testing crops for chemical tolerances was necessary because, although the chemotherapeutants are potentially useful for viral eradication, some have been reported to inhibit growth and, at certain concentrations, to be phytotoxic; it is also probable that some might be mutogenic (R. Hull, personal communication). For example, workers in CIAT (Columbia) found that cassava could not be grown on media containing Ribavirin (Dr. E. Rolando, personal communication).

4.4.2 2-Thiouracil

2-Thiouracil was found to be phytotoxic to sweet potato plantlets at all concentrations tested, so that no plants were recovered from the cultures. Mancino *et al.* (1984) reported that, while trying to eradicate cowpea chlorotic virus from cowpea (*Vigna unguiculata*), thiouracil was phytotoxic. Dawson (1984) also found thiouracil to be phytotoxic to tobacco (*Nicotiana tabacum*) plants cultured *in vitro*. However, Borissenko *et al.* (1985) reported 46.6% eradication of potato viruses from

potatoes without phytotoxicity occurring when 4 mg/l thiouracil was added to the culture medium. In these investigations, thiouracil at 5 mg/l was phytotoxic to sweet potato cultures. This indicated that, like cassava and possibly also cowpea and tobacco, thiouracil could not be used for chemotherapy with sweet potato.

4.4.3 Vidarabine

The observed physiological effects of Vidarabine on plant growth of sweet potato cultures are similar to those reported previously for other species. Dawson (1984) reported that it had antiviral effects on several plant viruses, but did not comment on its phytotoxicity. Stone (1982) reported the eradication of viruses from *Ullucus tuberosus* meristem-tips cultured on a culture medium containing 1 mg/l of Vidarabine, and further observed that at this concentration it stimulated plants growth; at 10 mg/l, however, Vidarabine was totally phytotoxic. My results support and extend this observation; thus, although Stone (1982) used concentrations of 1 and 10 mg/l, my experiments tested the effects of concentrations of 2, 4, 6, 8 mg/l. Vidarabine stimulated growth, the effects being optimal at a concentration of 4 mg/l; growth was inhibited at higher concentrations, as can be seen in Figures 53, 54a, 54b & 54c. At a concentration of 8 mg/l, plants were small and stunted. However, virus assays indicated that Vidarabine (Table 25) was relatively ineffective as a chemotherapeutant and so could not be recommended for use with sweet potatoes.

4.4.4 DHT and Ribavirin

The physiological effects of DHT and RB on the growth of sweet potato meristem-tip and shoot-tip cultures observed in my studies were similar to those reported previously. Generally, an increase in the concentration of chemotherapeutants resulted in a corresponding decrease in plant vigour as observed

in the reduction in internode numbers, plants length and fresh weight. Similar observations had been reported in other crops by Klein *et al.* (1982), Simpkin *et al.* (1981), Wambugu *et al.* (1985), Cassels *et al.* (1982) and Borissenko *et al.* (1985). However, sweet potato cultures were observed to tolerate far higher concentrations of RB and DHT than solanaceous species such as potato and tobacco. In my studies, the meristem-tip culture plantlets were regenerated from media containing 60 mg/l RB and 100 mg/l DHT. From shoot-tip cultures, plants were regenerated from media containing 150 mg/l RB or DHT, and from those with combinations of DHT 100 mg/l and RB 10 mg/l. Borissenko *et al.* (1985) reported that potato meristem-tip cultures failed to regenerate in media containing more than 100 mg/l DHT. Both Simpkin *et al.* (1981) and Klein *et al.* (1982) similarly reported that RB 100 mg/l was completely phytotoxic to potato tissue cultures. Most reports indicate that at 10 mg/l Ribavirin had little phytotoxic effects on most species but was reasonable effective in facilitating viral eradication from *in vitro* cultures (Wambugu *et al.*, 1985; Simpkin *et al.*, 1981; Klein *et al.*, 1982). Recent reports indicate that RB and DHT are now widely used to eliminate viruses from plantlets grown *in vitro*. Bova (1990) reported the successful elimination (64%) of plum pox virus from MTC of plum cultivars grown in media containing 10-20 mg/l RB; similarly, Bittner *et al.* (1990) reported the successful elimination of PVS, PVX and PVM from potato with DHT and RB. Deogratias *et al.* (1989) eliminated PDV, PNRSV, ACLSV from sweet cherries by a combination of chemotherapy (DHT, RB and Cyanoguanidine, 10-500 mg/l) and thermotherapy *in vitro*, and Albouy *et al.* (1988) successfully eliminated (95%) orchid viruses from *Cymbidium* spp. grown in media with RB (25 ppm). Griffiths *et al.* (1990) reported the reduction in concentration of PVS, PVM and PVX, PVY and potato leaf roll viruses in potato by the use of RB (20 mg/l) and alternating temperatures, that were relatively low (31 °C) in the light cycles.

Walkey (1980) also in a review on the production of virus-free plants by tissue culture, reported that, it was possible to eradicate viruses such as cucumber mosaic virus (CMV) and alfalfa mosaic virus (AlfMV), by culturing infected meristematic tissues at 30-40 °C (Walkey, 1976); high temperature apparently blocked virus replication and since virus degradation also occurred concurrently (Kassanis, 1957), the end result was complete virus eradication. The duration of the high temperature treatment was often critical to avoid virus resurgence that may occur due to inactivation of a virus resistance factor in the host; for CMV and AlfMV 45 days at 32 °C or 9 days at 40 °C, was required for virus eradication, but the duration of treatment may vary for other viruses (Walkey, 1976). The daily cycles of high and low temperatures were frequently preferable to continuous high temperature treatment, as they were less damaging to plant tissues (Walkey and Freeman, 1977).

4.4.5 Conclusions

Results from my studies indicate that 2-thiouracil and Vidarabine cannot be used as effective antiviral agents in MTC of sweet potatoes for the reasons already discussed. DHT also is an ineffective antiviral agent with STC of sweet potatoes. Although combinations of DHT and RB (RB 10 mg/l + DHT 100 mg/l) are effective in eliminating virus (71%), they could have serious deleterious effects on growth. This combination was observed in my other studies (6.3.2.5, 6.3.2.7) to facilitate plant regeneration from leaf derived callus, but did not eliminate viruses as reported by others (Shepard *et al.*, 1980; Simpkins *et al.*, 1981). RB alone at a concentration of 40 mg/l can be used to eliminate viruses from sweet potato MTC *in vitro*; thus, of the 55% of plantlets that survived, virus was eliminated from 67% (i.e. more than twice that of the controls 32%). From statistical analyses of the data for internode number, plant length and fresh weight of plantlets, there was no significant

differences between the effects of RB 40 & 10 and 0 & 10, results suggesting that 40 mg/l was the highest concentration having the least deleterious effect on growth. No obvious variants were detected in field observations of MTC sweet potato plants originating from RB treatments. However, although the field experiment utilizing RB and DHT derived plants were not properly randomized, the data obtained have given some notable indications. The data indicate that RB (40 mg/l) had a serious effect on the fresh weight of foliage and tubers that carried over to the field; the yields were reduced by 86% and 51% for foliage and tubers, respectively. The reduction of tuber yields is particularly important, especially if such a procedure were to be used to eliminate viruses from planting materials that were to be used soon after for cropping. MTC using DHT at 60 mg/l was overall the best treatment, effecting 65% virus elimination with 71% survival of plantlets. Statistical analysis of the effects of 60 mg/l DHT on growth, expressed as the number of internodes stem length and total fresh weight, indicated that at this concentration its effects were not significantly different from those at a concentration of 40 mg/l; it was, therefore, considered for sweet potato to be the highest concentration that had minimum deleterious effects on growth. At 60 mg/l, DHT had negligible effects on the total fresh weight produced by the foliage of field grown plants (only c. 2% reduction). However, it reduced the fresh weight of tubers by c. 34%. This suggests that DHT, like RB, had important effects that "carried over" to plants subsequently grown in the field. This subject would need to be further investigated if the two chemotherapeutants were to be more widely used. For example, it would be necessary to take cuttings from the affected field-grown sweet potatoes during the first cropping season and subsequent cropping seasons, to establish whether, and/or when, the "carry over" effects of DHT and RB were diminished.

CHAPTER 5

TISSUE CULTURE STUDIES: IN VITRO THERMOTHERAPY AS AN ADJUNCT TO MERISTEM-TIP CULTURE (MTC) FOR OBTAINING VIRUS-FREE SWEET POTATOES

5.1 INTRODUCTION

In vivo thermotherapy of meristem-tip culture (MTC) has been utilized for the production of virus-free plants of numerous vegetatively propagated species including sweet potato as described in the reports given in section 4.1. For sweet potato there are also several reports of regeneration procedures for obtaining virus-free plants, for germplasm conservation, propagation, and international exchange (section 4.1). However, the wider application of these techniques has been limited by certain difficulties resulting in low survival rates (20-40%), prolonged regeneration period (6-12 months) and the production of a low proportion of virus-free plants (20%) (Moyer (1988)). In order to improve the efficiency of MTC techniques, new adjunct methods, such as *in vitro* chemotherapy have been investigated (see 4.1).

Attempts to improve the efficiency of meristem-tip culture for obtaining virus-free sweet potato plants by *in vitro* thermotherapy are now described and discussed.

5.2 MATERIALS AND METHODS

5.2.1 *In vitro* thermotherapy

The methods for obtaining explants from the greenhouse grown plants (section 2.2) and for surface sterilization were as described in section 2.4. For each genotype, 50 lateral buds or 3-4 mm shoot tips were excised from the explants and each cultured

in a 30 ml universal bottle (Sterilin) containing 10-15 ml of MS basal medium (Table 6) supplemented as required for different developmental stages as described in Table 7. The cultures were incubated at 25 °C for three weeks before transfer to a growth chamber (Gallenkamp) for heat therapy at 30 °C with a photo-period of 16 hours (30 $\mu Mm^{-2}s^{-1}$ PAR). The temperature of the growth chamber was gradually raised by 2 °C per day, for the light cycle only, so that by the seventh day it had reached 40 °C. (Plants failed to survive at temperature above 40 °C). The cultures were then incubated in 40 °C, during the light period and 30 °C, during the dark period cycles for 4 weeks.

5.2.2 Meristem-tip Culture (MTC)

After heat therapy, the *in vitro* plantlets were removed from the growth chamber, placed on a filter paper as described in section 2.4 and meristems (0.5-1.0 mm) were excised for further culture. Five meristems were cultured on MS basal medium supplemented as for stage I (Table 7) contained in 5 cm deep-form Petri dishes (Sterilin) sealed with parafilm and incubated in the growth room at 25 °C, and 16 hr photoperiod (30 $\mu Mm^{-2}s^{-1}$ PAR). After one week, the MTC were transferred to fresh medium. After four weeks, all MTC were transferred to fresh stage I medium (table 7) and after a further eight weeks they were transferred to stage II (Table 7) medium for four weeks. After this transfer, any plantlet reaching 2 cm in height was removed from the 5 cm deep-form Petri dish, and transferred to 100 ml specimen container (Sterilin 185A) containing 15 ml MS basal medium (Table 6) supplemented as for stage III (Table 7). After four weeks, plantlets were transferred to 150 ml specimen containers (Sterilin 165A) containing similar medium but without agar; four plantlets, each supported on a Milcap plug (Milcap France), were transferred into each

container. The plantlets which had already developed roots, had the roots removed before this final transfer. All of these cultures were incubated in a growth room at 25 °C and 16 hr photoperiod (30 $\mu M m^{-2} s^{-1}$ PAR) for another four weeks.

5.2.3 Acclimatization to soil conditions

Cultures were removed from the containers, dipped in water containing 0.5% Benlate (PBI) and 0.5% Captan (Murphy), before being potted in 12.5 cm inch plastic pots, containing Fison C2 soil mixture. The plants were then covered with an inverted 500 ml clear polystyrene container which had the two bottom holes sealed with clear tape. The pots were placed on 4 cm plastic saucers, containing water 2 cm deep. The plants were placed on a bench in the greenhouse and illuminated using fluorescent tubes, with 16 hour photoperiod (30 $\mu M m^{-2} s^{-1}$ PAR). The plants were acclimatized by unsealing the bottom holes in the cover containers by the end of the second week, and they were uncovered at the end of the third week. An alternative acclimatization procedure involved planting the plantlets in a seed box with soil (Fison C2) and spraying them with water using a hand sprayer twice a day, and gradually opening the top vents. Both methods were used successfully to acclimatize the *in vitro* plantlets to greenhouse conditions. A summary of the culture, media, containers, place of incubation and time intervals involved to produce greenhouse plants through the *in vitro* thermotherapy and MTC system are given in Table 30.

Table 30 SUMMARY OF PROCEDURE FOR *IN VITRO* THERMOTHERAPY AND MERISTEM-TIP CULTURE (MTC).

Stages of development	Type of culture	Media MS (Table 6) + Supplements (Table 7)	Culture vessels	Incubation	Period of Incubation (weeks)
1	STC (3-4 mm)	Stage II	30 ml universal bottles	Growth room 25 °C	3
2	Plantlets	Stage III	Universal bottles	Growth chamber Thermotherapy °C	5
3	MTC (0.5-1.0mm)	Stage I	5 cm deep-form Petri dishes	Growth room	8
4	STC	Stage II	5 cm deep-form Petri dishes	Growth room	4
5	Plantlets	Stage II	100 ml specimen bottles	Growth room	4
6	Plantlets	MS-Liquid Stage III	150 ml specimen bottles with Milcap plugs	Growth room	4
7	Plantlets	Fisons C2 compost	5 inch pots or seedbox (acclimatization)	greenhouse	6

50 STC replicates per genotype for stage I.

78 MTC replicates for cv. Papota.

60 MTC replicates for genotype 157.

84 MTC replicates for cv. Rose Centennial.

Growth room conditions: 25 $°C$, 16 hour photoperiod (30 $\mu M m^{-2} s^{-1}$ PAR).

Growth chamber conditions as described in section 5.2.1.

Greenhouse conditions as described in section 2.2.

5.2.4 Virus Indexing

The virus assays with plants regenerated from *in vitro* thermotherapy with MTC were carried out by two methods: graft-inoculation (section 2.5.1.1) of fully acclimatized (5.2.3) greenhouse growing plants to *Ipomoea setosa*, and by ELISA (section 2.5.4.). 54 plants of cvs. Papota, 59 of RC, and 43 of genotype 157 were virus tested.

5.3 RESULTS

The experiment was undertaken to investigate the use and efficiency of in vitro thermotherapy as adjunct to meristem-tip culture (0.5-1.0 mm), in freeing sweet potato plants, from sweet potato feathery mottle virus (SPFMV) disease.

Three sweet potato cultivars Papota and Rose centennial, and genotype 157 obtained as described in section 2.1, and growing in the greenhouse under conditions described in section 2.2 were used as the source of explant. The culture procedures used for in vitro thermotherapy are described in details in section 5.2.1 and for meristem-tip culture in section 5.2.2. After two weeks, MTC for cv. Papota were forming basal callus, and they were transferred to the same medium but with 30 g/l sucrose, on which they resumed normal growth. The regenerated plantlets were

acclimatized to soil conditions as described as described in section 5.2.3; after which they were indexed for virus elimination (section 5.2.4).

The *in vitro* heat therapy technique favoured rapid and vigorous growth but temperatures above 40 °C killed the plantlets. It was necessary also to reduce the temperature of the dark cycle to 30 °C, to facilitate recovery of the plants. During the heat therapy the meristem grew faster than the leaves, and became the topmost projection leaving the leaves behind. This made the meristem excision procedure easier and the MTC from these in vitro plants also grew vigourously. The regenerated plants reached developmental stage five (table 30) and were about 2 cm tall after five months of culture. Full plantlet regeneration to the acclimatization stage was achieved in 7 months.

The detailed results for the rate of regeneration and virus indexing are given in Table 31. Overall for the three genotypes, 52 from the 74 MTC, were regenerated to greenhouse plants and 26 provided a virus-free assays. This represented an average regeneration rate of 70% and virus-elimination rate of 50%.

Table 31 IN VITRO THERMOTHERAPY FOLLOWED BY MERISTEM-TIP CULTURE (MTC) TECHNIQUE AND VIRUS ASSAY.

Genotype	No. of MTC	No. of MTC Regenerated and Virus tested	Rate of Regeneration (%)	Virus elimination rate (%)
Papota	78	54	69	54
157	60	43	71	46
Rose Centennial	84	59	70	49
Averages	74	52	70	50

5.4 DISCUSSION

Comparable reports of similar studies on *in vitro* thermotherapy as an adjunct to meristem-tip culture could not be found in the reported literature; however, there have been two recent reports of *in vitro* thermotherapy combined with chemotherapy. Deogratias *et al.* (1989) reported successful virus elimination of sweet cherry viruses (prune dwarf virus) (PDV), (prunus necrotic ringspot virus) (PNRSV) and (apple chlorotic leafspot virus) (ACLSV) from "micropropagation" cultures, by *in vitro* chemotherapy with Ribavirin, DHT and cyanoguanidine. They further showed that by subsequently submitting these plantlets to high temperatures (32-34 °C), it was possible to obtain 100% virus-free plants. Griffiths *et al.* (1990) reported that, chemotherapy with Ribavirin (20 mg/l) on potato nodal cultures, combined with thermotherapy alternating 4-hour cycle of 35 and 31 °C, for four weeks, was effective in reducing 10-60 fold the virus concentration of potato viruses M, S, and X, while that of potato Y and potato leaf roll viruses was reduced fourfold.

Walkey, (1976) reported successful eradication of cucumber mosaic virus (CMV) and alfalfa mosaic virus (AlfMV) by cultivating infected meristematic tissue

at 30-40 °C for 6-12 weeks, and further suggested that, there was a critical high temperature and duration of thermotherapy required for effective blocking of virus replication and degradation; and to avoid virus resurgence, but added that, this varied for different viruses. Walkey (1980) reported that the daily cycles of high and low temperatures were less damaging to the host tissues and that virus degradation following blocking of virus synthesis occurred at low temperatures. This compares with my studies of thermotherapy treatment of 40 to 30 °C daily cycles for four weeks, although in my case whole *in vitro* virus (SPFMV) infected plantlets were heat treated instead of meristem-tip cultures.

The apparent improvement in efficiency of *in vitro* thermotherapy of plantlets with MTC in comparison with reports of *in vivo* thermotherapy with MTC, could have been due to several reasons, including some of those discussed earlier. Probably the thermotherapy treatment of the *in vitro* plantlets had caused considerable virus degradation and blocking of virus replication, reducing the overall virus titre in the plantlet, and allowing more and bigger sized (0.5-1.0 mm) meristem-tips excised to be virus-free. This would consequently result in the improved regeneration rate (70%), regeneration period (7 months) and virus-free assay (50%). Also, during the thermotherapy period, the meristem tips had grown so rapidly that they had become the uppermost projections of the plant, were not covered by leaves, and were above the leaf primordia. This meant that during excision, relatively larger meristem-tips (0.8-1.0 mm) could be taken with minimum damage and with less risk of virus contamination from the more mature part of the plantlet. Further, the direct culture technique without surface sterilization ensured less damage, minimal dehydration and contaminations of the meristem tip during the culture procedure.

Moyer (1988) suggested that the inefficiency of the direct meristem-tip culture procedure was the result of contamination, mortality due to the small size,

dehydration during the culture procedure, and the lack of regeneration resulting from genotypic differences. It seems that the combined *in vitro* thermotherapy of plantlets and MTC procedures were able to overcome most of these problems.

Overall, the published results and the results from this investigation indicate that *in vitro* thermotherapy combined with the MTC technique is a good alternative to *in vitro* chemotherapy combined with MTC; and that either are a considerable improvement over *in vivo* thermotherapy with MTC. *In vitro* thermotherapy combined with the STC technique, although having a high rate of regeneration was not as effective for virus elimination.

The apparent improvement in efficiency of *in vitro* thermotherapy with MTC in comparison with reports of *in vivo* thermotherapy with MTC, could have been due to several reasons. During the thermotherapy period, the meristem tips had grown so rapidly that, they had become the uppermost projections of the plant, and they were not covered by leaves. This meant that during excision, relatively larger meristem-tips (0.8-1.0 mm) could be taken with minimum damage and with less risk of virus contamination from the more mature part of the plantlet. Also the direct culture technique without surface sterilization ensured less damage, minimal dehydration and contamination of the meristem tip during the culture procedure. Moyer (1988) suggested that the inefficiency of the direct meristem-tip culture procedure was the result of contamination, mortality due to the small size, dehydration during the culture procedure, and the lack of regeneration resulting from genotypic differences. It seems that the combined *in vitro* thermotherapy and MTC procedures were able to overcome most of these problems.

CHAPTER 6

STUDIES ON SWEET POTATO REGENERATION FROM CELL CULTURES

6.1 Introduction

It is now widely accepted that plant-cell and tissue culture technique, especially those associated with genetic manipulation procedures, will have an increasingly important role in plant improvements (Henshaw *et al.*, 1982; Cocking, 1987; Fraley, 1989 and Brown *et al.*, 1987). Difficulties, however, are often encountered in regenerating plants from cells or tissues that have been successfully manipulated and this can be one of the major factors limiting progress in this field.

In crops such as tobacco and potato, which have received much scientific interest, an extensive literature exists and successful regeneration procedures have been developed, and the systems are being utilized effectively for genetic manipulations and crop improvement (Sandra *et al.*, 1984; Nelson *et al.*, 1988; Secor *et al.*, 1981; Shepard *et al.*, 1977). By contrast, there have been fewer reports concerning sweet potato, especially with mesophyll and protoplast cell cultures, and most of them describe isolation of cells and callus colony formation but no regeneration of plants (Wu *et al.*, 1979; Bidney *et al.*, 1980; Otoni *et al.*, 1987; Schwenk *et al.*, 1981). Successful, however, plant regeneration from sweet potato protoplast and other cell cultures has recently been reported by several workers.

Sihachakr *et al.*, (1987) reported plant regeneration from protoplast cultures, but with only a 5% success rate with one genotype. Murata *et al.*, (1987) reported successful plant regeneration from petiole-derived protoplasts, on MS basal medium supplemented with hormones, and then in 1989 Murata *et al.*, reported further

improvement in regeneration by supplementation of the medium with, glutamic acid, asparagine and proline. Chee *et al.*, (1989) reported successful high frequency embryo development from discrete cell aggregates (250-355 μm.) cultured on solid medium, and from cell aggregates anchored on alginate beads; these authors suggested that the improvement in regeneration was in response to structural polarity since such development was not achieved beyond the globular stage in cell aggregates cultured in suspension cultures.

Plant regeneration from tissue and organ-derived callus cultures (leaf, root, anther, ovule, cotyledons etc.) of sweet potato have, in comparison, been better studied and reported. The regeneration of plants from anther culture has received particular attention because most sweet potato cultivars are self sterile and the production of in-bred lives is difficult (Tsai *et al.*, 1973; Tsai, 1973; Yamaguchi, 1978); considerable success has been reported by Sehgah (1978) and Tsai *et al.*, (1979). More recently, Xue (1987) reported successful plant regeneration from callus derived from plumular axes and radicles of sweet potato cotyledons, by use of MS basal medium with hormones, but draw attention to the strong genotypic difference. Xue (1988) also reported successful organ formation and plantlet regeneration from unfertilized ovule-derived callus cultures of *Ipomoea batatas* and its wild relatives *I. trifida* (6x) and *I. littoralis* (4X). Liu *et al.* (1990) reported successful plant regeneration in high frequencies, from callus derived from stem (68.4%), petiole (42.1%), and leaf (55%) explants, by use of MS basal medium supplemented with hormones, but noted that whole plantlet development occurred only in the final hormone-free medium stage.

Somatic embryogenesis has also been reported in sweet potato from leaf discs (Liu *et al.*, 1984), leaf-derived callus (Sehgah, 1975), stem, leaf and storage root explants (Carswell *et al.*, 1984), axillary buds (Jarret *et al.*, 1984) and root discs

(Hwang *et al.*, 1980). Schulthesis *et al.* (1988) reported successful plant regeneration from somatic embryos in gel carriers and indicated the strong influence of the carbon source supplement. Dewald *et al.* (1988) also reported on the study of the histology of callus initiation and somatic embryogenesis from sweet potato shoot apices and observed that highly embryogenic callus was initiated from the meristematic regions of the shoot apices, while non-embryogenic callus was formed from the older leaf primordia of the shoot apices. Chee *et al.* (1988) reported successful selective enhancement of embryogenic and non-embryogenic callus growth in sweet potato by manipulation of hormones and the use of liquid or solid media. Chee *et al.* (1988) in another paper reported further improvement in somatic embryogenesis and plant regeneration resulting from three different stages of development late torpedo, cotyledonary stages and from precocious hypocotyl expansions. Chee *et al.*, (1989) in a study on the mechanism by which the development from embryogenic callus to embryo development was inhibited, showed that the added exogenous auxins disrupted the efflux of the endogenous IAA from embryogenic loci, results probably explaining why in most cases with sweet potato, embryogenesis and or plantlet regeneration occurs only in hormone-free medium. Chee *et al.* (1990) reported further improvement in somatic embryo recovery in sweet potato by manipulation of the hormones and the level of sucrose.

Although, however, reasonable advances have been made recently to improve both organogenesis and embryogenesis in sweet potato, most reports indicated relatively limited success and strong genotypic influence, indicating that further studies are still required to develop both media and culture conditions for widespread use.

The main research objective of this study was to improve further the

methodologies of plant regeneration from cell culture systems (leaf cells, leaf, and root derived callus cultures) for widespread use, as a prerequisite for future genetic manipulations and/or viral elimination.

6.2 MATERIALS AND METHODS

6.2.1 Root Cultures

Pieces of stem vines from the greenhouse plants were used as the source of root explants and the surface sterilization procedures were as described in section 2.4. Root-tips at the leaf internodes, that were still covered by the epidermis, were used to initiate the root-cultures. After surface sterilization, the epidermis was peeled off using fine forceps, and the root tips (4 mm) were excised and placed in the liquid medium in a 100 ml sterile specimen container (Sterilin 18.5A). The root-tip cultures were incubated in an illuminated orbital shaker (Gallenkamp) at 100 revolutions per minute, 25 °C and 16 hours photoperiod (30 $\mu Mm^{-2}s^{-1}$APR) for 2-3 weeks.

The medium for root-culture initiation was composed of half strength MS (Table 6) supplemented as for stage III (Table 7) with hormones G_1A_3 10 mg/l and Kn 0.4 mg/l but without agar.

After three weeks, the root cultures which had elongated to over 10 cm and produced lateral roots were removed from the liquid medium and used as the source of explants for the studies.

6.2.2.1 Leaf Culture

6.2.2.1 Callus Induction from Leaf-explant

The second fully expanded leaves from the growing tips of the sweet potato genotypes in the greenhouse were used as sources of explants. These were surface sterilized as described in section 2.4; using sterile surgical blades (Swann Morton

Ltd.), forceps and Petri dishes, small leaf sections 10 mm square were cut, avoiding the mid-rib and edges, and laid flat in contact with the media, abaxil side downwards. Ten pieces of leaf sections were cultured on each Petri dish containing 20 ml MS basal medium (Table 6), supplemented as for stage III (Table 7), (minus GA_3) and with the concentration of 2,4-D; 0.1, 0.3, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/l. Ten Petri dishes replications were used for each genotype and for each 2,4-D test concentration. All Petri dishes were wrapped in parafilm (Parafilm M-USA), and incubated at 27 °C in a growth incubator (GallenKamp Co. Ltd.) in the dark, for three weeks.

6.2.2.2 Callus Induction from *in vitro* Shoots

Stem pieces were obtained from greenhouse and sterilized as described in section 2.4. The lateral buds were removed and cultured in 9 cm Petri dishes on MS basal (Table 6) supplemented for stage II (Table 7). Ten Petri dishes, each containing four axillary buds, were cultured for each genotype, wrapped in parafilm and incubated in the growth room in the conditions described in section 2.4, for three weeks. After three weeks, 7 mm long shoots with two fully expanded leaves (5 mm long), that have developed from the axillary buds were transferred to the different 2,4-D containing media as described in section 6.2.2.1 and incubated in the growth chamber (GallenKamp) at 27 °C, in the dark, for three weeks.

6.2.2.3 Regeneration Procedures

MS basal medium (Table 6) either full or half strength, supplemented as for stage I (Table 7) minus GA_3, was used throughout the regeneration studies. The media were prepared as described in section 2.3, with all hormones added before autoclaving, except for IAA which was filter sterilized by use of a syringe (Gillette) and membrane filter (0.2 mm Gelman Disposable Filter), and added to the cooled

media. 20 ml of the different media were dispensed into 9 cm Petri dishes and labelled. Five pieces of callus approximately 5 mm in diameter were transferred from the relevant callus induction medium, to 20 ml of the different regeneration medium in 9 cm Petri dish replications were used for each genotype, giving each test 50 callus replication. Cultures were incubated in the growth room at 25 °C and 16 hr photoperiod (30 $\mu M m^{-2} s^{-1}$APR).

Cultures in medium with 1AA were covered a neutral density filter box for two weeks before being exposed to full light intensity. During the incubation period cultures were observed for regeneration responses at weekly intervals over a period of four weeks before transferred to hormone-free MS basal medium with double sucrose concentration (60 gm/l).

6.2.2.4 Testing for Virus Elimination

Plantlets regenerated from leaf derived cultures, and growing in the greenhouse under conditions described in section 2.2, were tested for virus infections using the DAS-ELISA technique as described in section 2.5.4.

6.3 RESULTS

6.3.1 Root Culture Regeneration

The objective of the experiment was to investigate plant regeneration from root-derived callus cultures.

Three sweet potato genotypes Papota, HIB 687, and Brondal, obtained as described in section 2.1 and maintained in the greenhouse as described in section 2.2 were used for the study. The culture methods used are described in section 6.2.1.

Three weeks old root cultures of Papota Brondal and HIB 687 (section 6.2.1) were removed from the liquid medium using long forceps and placed in 9 cm heat sterilized glass Petri dish (Pyrex) containing a sterile filter paper (Whatman No. 1). They were then cut into 1 cm long pieces each with a single lateral root (Fig 60a), and cultured in 9 cm Petri dishes (Sterilin) on 20 ml solid MS basal medium supplemented as for stage III (Table 7), and with the hormones and/or chemotherapeutants being tested. Ten root pieces were cultured per Petri dish, with ten Petri dish replications per treatment. The cultures were sealed with parafilm and incubated in the growth room under the conditions described in section 2.4. Cultures in test media with 1AA were first converted with neutral density filter box for two weeks before being exposed to full light. Cultures were incubated for 6 weeks for regeneration responses. The hormone and chemotherapeutant combinations tested for regeneration potential are shown on Table 32.

Results from the different experimental treatments are summarised in Table 32. Generally, the hormones or hormone combinations with DHT or RB produced some very distinctive morphological responses with strong genotypic influences, but no plants were regenerated. Zn/IAA stimulated the formation of green callus which looked as if it might have some regeneration potential, but no plants were produced. BA/NAA or 1AA alone produced creamy to white friable callus. DHT alone

stimulated numerous root development while combinations with RB or adenine proved inhibitory to root-growth, except for genotype HIB 687. Combinations of Zn, 1AA with either DHT or RB, produced callus, while the four chemicals combination produced both callus and root. High sucrose (60 g/l) produced numerous root growth except for Papota. Only kinetin 0.2 mg/l and 0.4 mg/l was observed to stimulate plant regeneration from primary callus, derived from the cut edges of the original explant of one genotype (HIB 687, Fig 60b) after 8 weeks of culture. On average two plants were regenerated out of the 10 original root culture explants per Petri dish, giving a regeneration rate of 25%.

Table 32. EFFECTS OF DIFFERENT COMBINATIONS OF HORMONES, RB AND DHT, ON MORPHOGENETIC RESPONSES OF ROOT CULTURES

Media (mg/l)	Genotype	Morphogenetic Response
Kn 0.2	Papota	Lateral roots ending in swellings
	Brondal	No lateral roots or callus
	HIB 687	Poor-lateral roots, callus formation from edges of original explant followed by shoot regeneration
Kn 0.4	Papota	Explant edges producing callus, and lateral roots
	Brondal	Explant edges producing callus, no lateral roots
	HIB 687	Explant edges producing callus, and numerous lateral roots followed by shoot regeneration
Zn 0.25 1AA 0.50	HIB 687 Papota Brondal	Green callus
BA 0.1 NAA 1.0	Papota Brondal HIB 687	Creamy growing callus
Rb 10	Papota	Callus and few lateral roots
DHT 100	Papota	Numerous lateral roots
DHT 100+ Rb 10	Papota	Poor lateral roots growth
Ad 20 DHT 100	Papota Brondal HIB 687	Poor lateral roots Numerous root growth & branching
Ad 20 DHT 100 Rb 10	Papota Brondal HIB 687	Lateral roots Lateral roots Lateral roots and callus

Table 32. (Continued)

Media (mg/l)	Genotype	Morphogenetic Response
Zn 0.25 1AA 0.50 RB 10.0	Brondal HIB 687 Papota	Green compact growing callus
Zn 0.25 1AA 0.50 DHT 100	Papota Brondal	Green, nodulating compact callus
Zn 0.25 1AA 0.50 Rb 10.0 DHT 100	Papota Brondal HIB 687	Lateral roots
1AA 0.5 Rb 10 DHT 100	Papota HIB 687 Brondal	White callus at explant edges
BA 0.1 NAA 1.0 DHT 100 Rb 10	Papota Brondal HIB 687	Creamy callus
MS+60 gm sucrose	Papota HIB 687 Brondal	Few lateral roots Long branching green lateral roots with swollen tips Numerous green roots with short branching lateral roots

Medium: MS basal supplemented 3% (w/v) sucrose.

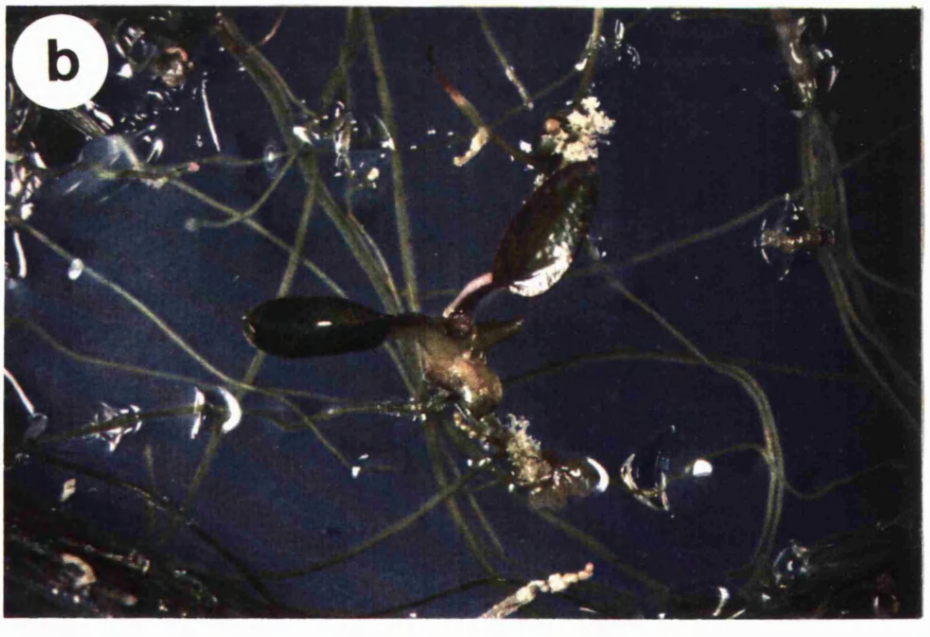
Replicates: 100 per genotype per treatment.

Incubation period: 6 weeks.

Incubation conditions: as for Table 30.

Fig·60 Root culture on MS medium supplemented with 3% (w/v) sucrose and 0.2 mg/l Kn, incubated in the growth room (25 °C, 16 hr photoperiod 30 $\mu Mm^{-2}s^{-1}$PAR).

a. Explant at zero day (Mag x 7).



b. Plantlet after culture for eight weeks (Mag x 1).

6.3.2 Leaf Culture Regeneration

6.3.2.1 Callus Induction

The experiment was conducted to investigate the use of 2,4-D for callus induction from *in vitro* leaf explants and from *in vitro* shoot explants, of different sweet potato genotypes. Various combinations of hormones and chemotherapeutants subsequently were then tested for their effects on plant regeneration from the callus and viral elimination. The sweet potato genotype used included HIB 687, 157, 512, KS 589, and cultivars Papota, Rose Centennial and Jasper. The methods used for inducing callus from the leaf and *in vitro* shoot explants are described in sections 6.2.2.1 and 6.2.2.2 respectively. The concentration of 2,4-D tested for callus induction included 0.1, 0.3, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/l.

After two weeks most of the leaf sections had produced callus and, depending on the genotype and 2,4-D concentration, some callus had started growing. After three weeks of culture incubation, there was complete callus induction, for both leaf and *in vitro* explants, for all genotypes, and in all 2,4-D concentrations (see Table 33), however, the rate of callus growth, texture, colour, quality and quantity was genotypically dependent, and optimal responses occurred at different 2,4-D concentrations. *In vitro* cultures performed better than the leaf cultures in all cases, but the trend of responses in relation to 2, 4-D concentration was the same for both types of explants, and for all the genotypes. The callus from optimum induction (++++) was used for subsequent regeneration experiments.

Table 33. EFFECT OF 2,4-D ON CALLUS INDUCTION FROM LEAF SECTIONS FROM DIFFERENT SWEET POTATO GENOTYPES

2,4-D Conc. (mg/l)	0.1	0.3	0.5	1.0	1.5	2.0	2.5	3.0
Papota	+	++++	+	+	+	+	+	+
HIB 687	+	++	+++	++++	+++	++	++	++
157	+	++	++	+++	++++	+++	++	++
512	+	+	++	+++	+++	++++	++	++
R.C.	+	++	++++	++	+	+	+	+
Jasper	+	+	++	++++	++	+	+	+
KS 589	+	+	+	+	++	+++	++++	+++

\+ Callus induction but no further growth

++ Callus induction with some limited growth

+++ Callus induction followed by good growth

++++ Optimum callus induction followed by prolific growth

Medium: MS-basal + 30 g/l sucrose

Explants: 8X10 mm leaf pieces or *in vitro* shoots

Replicates: 100 per treatment

Incubation conditions: 27 °C, in the dark, for three weeks.

6.3.2.2 Shoot Regeneration By use of Hormones BA/NAA systems

The combinations of hormones and basal media tested for their effects on shoot regeneration, from the leaf callus of the different sweet potato genotypes (as described in section 6.3.2.1 and summarised in Table 33) are listed in Table 34.

MS basal medium (Table 6), supplemented as described for stage I (Table 7) but without the GA_3 was used. The media were prepared as described in section 2.3, and all hormones except 1AA described in Table 34 were added before autoclaving, 1AA was filter sterilized and added to the cooled medium; 20 ml aliquots of the different

media were distributed into 9 cm Petri dishes. Five pieces of five mm callus (Fig 61a) cultures from callus induction medium were transferred into ten Petri dish replicates of regeneration media (Table 34), and incubated in the growth room under conditions described in section 2.4, for six weeks. Other culture techniques including final transfer to high sucrose (60 g/l) hormone-free MS basal medium were like those described in section 6.2.2.3.

The summary of the results from the different treatments is given in Table 34 and Figs 61a-d. Generally for all genotypes the callus responded with some kind of growth. The genotypic differences in response to the hormones were very pronounced; thus HIB 687 on treatments R1, R2, R3, R4, R7 and R8; and on final transfer to MS with 60 g/l sucrose, showed the most vigorous morphogenetic response expressed as callus nodulation (Fig 61a), and later by the formation of red nodule-like structures (Fig 61b) which developed into vertical root-like structures (Fig 61c) ending in tiny swellings. When HIB 687 callus was transferred from R1 (NAA 1.0 mg/l + BA 0.1 mg/l + GA_3 10.0 mg/l) to MS basal with 60 g/l sucrose, tiny shoot buds (Fig 61d) developed from the base of the green vertical root-like structures. The shoot buds developed further into independent shoots loosely attached to the root complex and after a further two weeks they were removed and cultured on MS supplemented with 30 g/l sucrose where they developed roots and grew into whole plantlets. About 40% of the callus cultures from R1 treatment of HIB 687 regenerated shoots, which developed into plantlets. All other genotypes developed some kind of nodules on the different test media which when transferred to MS basal medium developed some adventitious roots, with terminal swellings, but no shoots were regenerated.

Table 34. EFFECTS OF DIFFERENT HORMONE COMBINATIONS ON THE MORPHOGENETIC RESPONSES OF SWEET POTATO CALLUS

			Morphogenetic Response	
Media Ref	Media Composition	Genotype	Hormone Media	MS+60 g/l sucrose
R1	MS FS NAA 1.0 mg/l BA 0.2 mg/l GA_3 10 mg/l	HIB 687	callus & red nodules	roots and shoot buds
		All other genotypes	callus	callus
R2	MS HS NAA 1.0 mg/l BA 0.1 mg/l GA_3 10 mg/l	All genotypes	As for R1 above but less vigour	As for R1 above but less vigour
R3	MS FS NAA 0.5 mg/l Kn 2.0 mg/l	HIB 687	callus & red nodules	a few roots but well formed terminal swellings
		All other genotypes	callus	callus
R4	MS HS NAA 0.5 mg/l Kn 2.0 mg/l	HIB 687 157	callus & nodules	a few roots
		All other genotypes	callus	callus
R5	MS FS Kn 0.2 mg/l	All genotypes	callus	callus
R6	MS FS Kn 0.4 mg/l	All genotypes	callus	callus

			Morphogenetic Response	
Media Ref	Media Composition	Genotype	Hormone Media	MS+60 g/l sucrose
R7	MS FS Ad 20 mg/l	HIB 687	callus & nodules	roots and terminal swellings
		All other genotypes	callus	callus
R8	MS FS Ad 20 mg/l	HIB 687	callus & nodules	few roots and terminal swellings
		All other genotypes	callus	callus
R9	MS FS Zn 0.25 mg/l IAA 0.5 mg/l	Papota HIB 687	callus & nodules	roots and terminal swellings
		All other genotypes	callus	callus

Medium: MS basal full strength (FS) or half strength (HS) supplemented with 3% (w/v) sucrose.

Replicates: 50 calluses per treatment.

Incubation condition: 25 °C, 16 hr photoperiod (30 $\mu Mm^{-2}s^{-1}$APR) for six weeks.

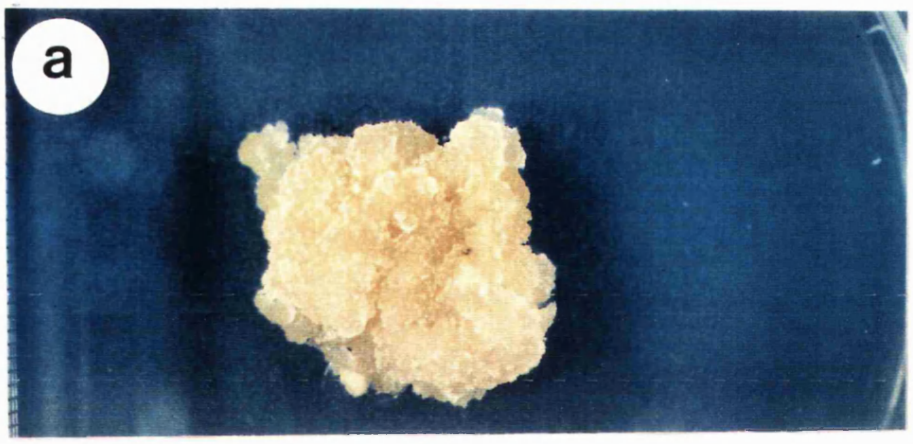
Fig. 61. Shoot regeneration from leaf-derived callus using BA/NAA hormone system of cv. HIB 687.

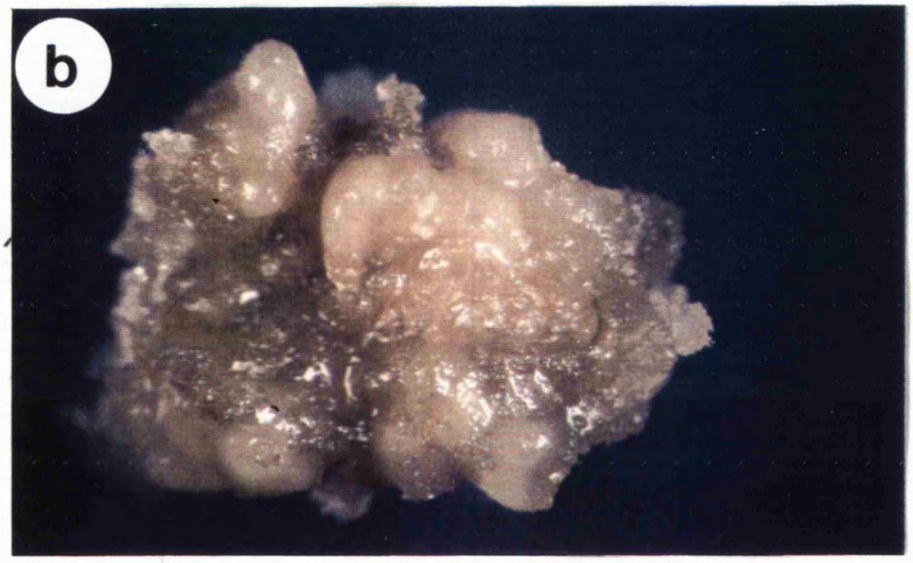
a. Callus formed after 3 weeks of culture on MS medium supplemented with 3% (w/v) sucrose and 1.0 mg/l 2,4-D, incubated in the growth cabinet (27 °C in the dark) (Mag x 2).

b. Nodule formation 2 weeks after transfer of callus to MS medium supplement with 3% (w/v) sucrose, NAA 1.0, BA 0.1 and GA_3 10.0 mg/l, and incubated in the growth room (25 °C, 16 hr photoperiod 30 $\mu Mm^{-2}s^{-1}$PAR) (Mag x 4).

c. Further development of the nodules after 3 weeks of culture on the same medium (Mag x 3).

Fig. 61





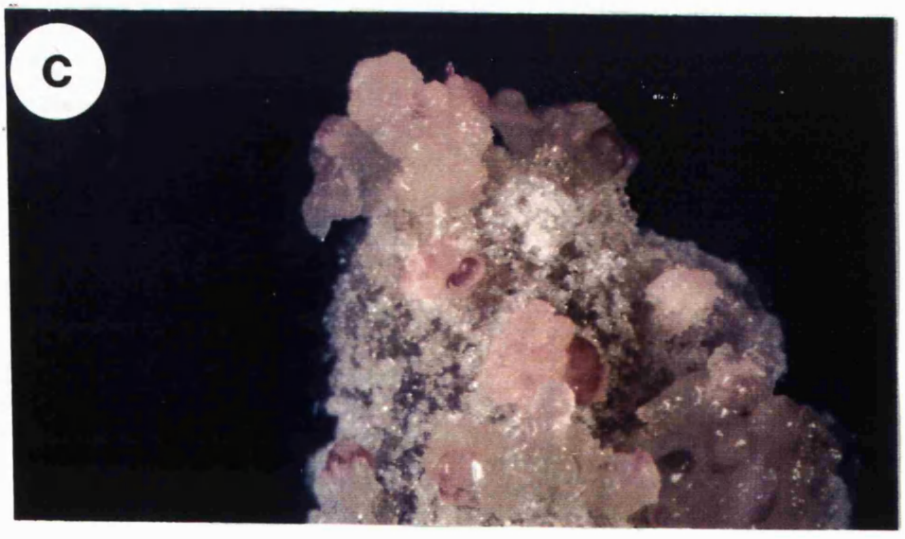
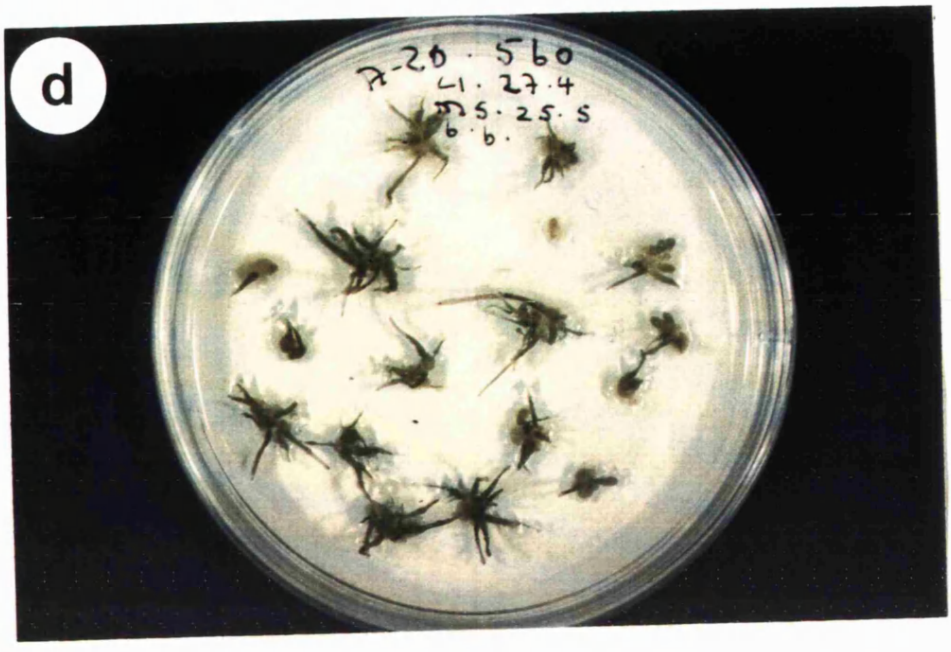


Fig.61



d. verticle root-like structures (adventitious roots) development from nodules after 4 weeks of culture on the same medium, ending with tiny pinkish swellings (Mag x 1).

e. Shoot-buds development at the base of adventitious roots 6 weeks after transfer to MS-medium (hormone-free) supplemented with 6% (w/v) sucrose (Mag x 3).

Summary of procedures leading to plant regeneration

The overall regeneration system produced plantlets in four stages:

Stage I: callus induction on 1.0 mg/l 2,4-D + MS + 30 g/l sucrose, 3 weeks incubation.

Stage II: vertical root-like structures incubation on R1 medium (MS + 30 g/l sucrose + NAA 1.0 mg/l + BA 0.1 mg/l + GA_3 10.0 mg/l); 4 weeks incubation (Fig 61c).

Stage III: shoot buds development on MS + 60 g/l sucrose; 6 weeks incubation (Fig 61d).

Stage IV: plantlet development on MS 1/2 strength + 50 g/l sucrose or MS + 30 g/l sucrose; 8 weeks incubation for production of a plantlet 3 cm long. The system produced plantlets that could be potted in 6 months.

6.3.2.3 Effects of Hormones and Chemotherapeutants on Regeneration and Virus Elimination

6.3.2.3.1 Effects of Chemotherapeutants on callus formation

Callus from different sweet potato genotypes (Papota, 512, 157, Jasper, KS 589 and HIB 687) were obtained as described in section 6.3.2.1 and summarized in Table 33.

MS basal medium (Table 6), supplemented as described for stage I (Table 7) but without the GA_3 and putrescine, was used. The media were prepared as described in section 2.3, and all chemotherapeutants described in section 4.2.2 were filter sterilized and added to the autoclaved and cooled media; 20 ml aliquots of the different media were distributed in 9 cm Petri dishes. Other culture techniques, including transfer of callus from induction media to media with chemotherapeutants, conditions of

conditions of incubation, observations, and the final transfer to high sucrose (60 g/l) hormone-free MS basal medium, were like those described in section 6.2.2.3.

The chemotherapeutants combinations that were tested included:

R10 - RB 10 mg/l

R11 - RB 20 mg/l

R12 - DHT 100 mg/l

R13 - DHT 100 mg/l + RB 10 mg/l

R14 - 2-Thiouracil 10 mg/l

R15 - 2-Thiouracil 10 mg/l + VID 25 mg/l

R16 - VID 25 mg/l

R17 - VID 50 mg/l

R18 - VID 0.03 mg/l + RB 0.22 mg/l

For most genotypes, transfer of callus to media with chemotherapeutants resulted into browning and death of callus. However, for genotype HIB 687, on treatment R12 (DHT 100 mg/l) good callus growth was observed, and on R13 (DHT 100 mg/l + RB 10 mg/l) numerous green adventitious roots, some ending in swellings developed. These roots continued to grow when transferred to hormone-free MS basal medium.

6.3.2.3.2 Effects of Combinations of hormones and Chemotherapeutants in single stage application on callus production, Regeneration and Virus Elimination

Callus from genotyps HIB 687, Papota, and 157 obtained as described in section 6.3.2.1 and summarized in Table 33 were used for the study. The callus procedures were as described in section 6.2.2.3 and calluses were transferred directly to media containing the following combinations of hormones and chemotherapeutants:

R19- Zn 0.25 mg/l + 1AA 0.5 mg/l + RB 10 mg/l.

R20- Zn 0.25 mg/l + 1AA 0.5 mg/l + DHT 100 mg/l.

R21- Kn 0.2 mg/l + DHT 100 mg/l + RB 10 mg/l.

R22- Kn 0.4 mg/l + DHT 100 mg/l + RB 10 mg/l.

R23- NAA 1.0 mg/l + BA 0.1 mg/l + DHT 100 mg/l.

R24- Ad 20 mg/l + DHT 100 mg/l.

R25- Kn 0.2 mg/l + DHT 100 mg/l.

R26- Kn 0.4 mg/l + DHT 100 mg/l.

R27- Ad 20 mg/l + DHT 100 mg/l + RB 10 mg/l.

R28- 1AA 0.5 mg/l + RB 10 mg/l + DHT 100 mg/l.

R29- BA 0.1 mg/l + NAA 1.0 mg/l + GA_3 10 mg/l + DHT 100 mg/l + RB 10 mg/l.

The cultures were incubated in the growth room for four weeks before being transferred to high sucrose (60 gm/l) hormone-free MS basal medium, and incubated for another four weeks.

Generally most genotypes produced callus only without further developments

on most test media treatments. cv. Papota developed a few roots, some ending in swellings, whereas genotype HIB 687 developed numerous green adventitious roots, similar to but more vigorous than those observed in section 6.3.2.3.1.

6.3.2.3.3 Effects of Chemotherapeutants and hormones following two further stages

Genotypes that had responded in a promising fashion in the experiments described in sections 6.3.2.3.1 and 6.3.2.3.2, respectively, were subjected to two further stages running concurrently, and the effects on regeneration and viral elimination were investigated.

The media preparation, culture procedures and conditions of incubation were as described in section 6.2.2.3; calluses were obtained as described in section 6.3.2.1 and summarised in Table 33.

The most promising calluses from genotype HIB 687, selected on the basis of good colour and growth, were transferred from chemotherapy treatment described in section 6.3.2.3.1 as treatments R12 (DHT 100 mg/l) or R13 (DHT 100 mg/l + RB 10 mg/l) and transferred to hormone treatment medium R9 (Zn 0.25 mg/l + IAA 0.5 mg/l) (see Table 34). The cultures were incubated for four weeks on R9 medium, with regular weekly observations; were then transferred to high sucrose (60 g/l) hormone-free medium and incubated for a further 6 weeks; finally they were transferred to either MS basal medium with 30 g/l sucrose or half strength MS basal medium with 50 g/l sucrose.

The detailed results of the morphogenetic responses observed on callus after the incubation periods of the different regeneration stages are summarised on Table 35 and Figs 62a-62f. These results indicated that DHT 100 mg/l + Ribavirin 10 mg/l or DHT 100 mg/l alone, or after further transfer to high sucrose (60 g/l) medium

did not effect shoot regeneration from callus but stimulated the development of adventitious roots. Zn (0.25 mg/l) combined with 1AA (0.5 mg/l) and with or without further transfer to high sucrose, had no morphogenetic effect on callus.

The transfer of callus from DHT alone (stage I, Table 35) to Zn + 1AA medium (stage II, Table 35) did not have an immediate effect, but on further transfer to the high sucrose medium induced the formation of reddish nodules which developed into adventitious roots and shoots; about 40% of the callus regenerated shoots. The transfer of callus from the combined DHT + RB medium (stage I, Table 35) to Zn + 1AA medium (stage II, Table 35), resulted in the formation of numerous reddish nodules, some of which resembled dormant shoot buds (Fig 62c). The nodules did not develop further on this medium but when transferred to the high sucrose (60 g/l) medium (stage III, Table 35), they produced numerous shoot-buds, with some entire calluses switching to vigorous shoot regeneration (Figs 61d & 61e), after six weeks of incubation.

The shoot-buds developed directly from the callus and they were not associated with root formation as in the case of the regeneration described in section 6.3.2.2; a few roots were formed later after regeneration and occurred independently from shoot buds. The shoot-buds grew vigorously with only loose attachment to the callus reaching about 10 mm in length after six weeks. Shoot-buds 10 mm long (Fig 62e) were detached from the callus and transferred to MS basal medium (Table 6) supplemented as for stage II (Table 7), where they developed roots within two weeks, and grew to vigorous plantlets, reaching 3 cm in height after eight weeks (Fig 62f). Plantlets were acclimatized (see section 5.2.3), potted into soil and transferred to the greenhouse.

Table 35. SUMMARY OF RESPONSES OBSERVED DURING SHOOT REGENERATION FROM LEAF-DERIVED CALLUS USING CHEMOTHERAPEUTANTS HORMONES AND HIGH SUCROSE CONCENTRATION

Stage I		Stage II	Stage III	
DHT 100 mg/l	DHT 100 mg/l RB 10 mg/l	Zn 0.25 mg/l 1AA 0.5 mg/l	Sucrose 60 g/l	Responses
-	-	-	-	Callus (control)
+	-	-	-	Callus
+	-	-	+	few roots
-	+	-	-	few roots
-	+	-	+	numerous roots
-	-	+	-	Callus
-	-	+	+	Callus
+	-	+	-	Callus
+	-	+	+	roots, 40% calluses formed shoots
-	+	+	-	numerous nodules/dormant shoot-buds
-	+	+	+	numerous shoot buds, 80% calluses produced vigourous shoots.

Inoculum: calluses from section 6.3.2.1 (Table 33) of genotype HIB 687.

(+) transferred on medium

(-) not transferred on medium

Basal medium: MS + 30 g/l sucrose (60 g/l sucrose in stage III).

Replicates: 40 calluses (size 10 mm) per treatment.

Incubation conditions: 25 °C, 16 hr photoperiod (30 $\mu Mm^{-2}s^{-1}$PAR)

Stages I & II 4 weeks each, stage III 6 weeks incubations.

Fig. 62. Plantlet regeneration from leaf-derived callus using chemotherapeutants (RB + DHT) and Zn/IAA hormone system, cv. HIB 687.

a. Callus induction, same as Fig. 61a (Mag x 2).

b. Nodule formation 4 weeks after transfer of callus to MS medium supplemented with 3% (w/v) sucrose, RB 10 mg/l and DHT 100 mg/l, and incubated in the growth room (Mag x 4).

c. Further development of nodules and dormant shoot-buds 4 weeks after transfer to MS medium supplement with 3% (w/v) sucrose, Zn 0.25 mg/l and IAA 0.5 mg/l (Mag x 4).

Fig. 62

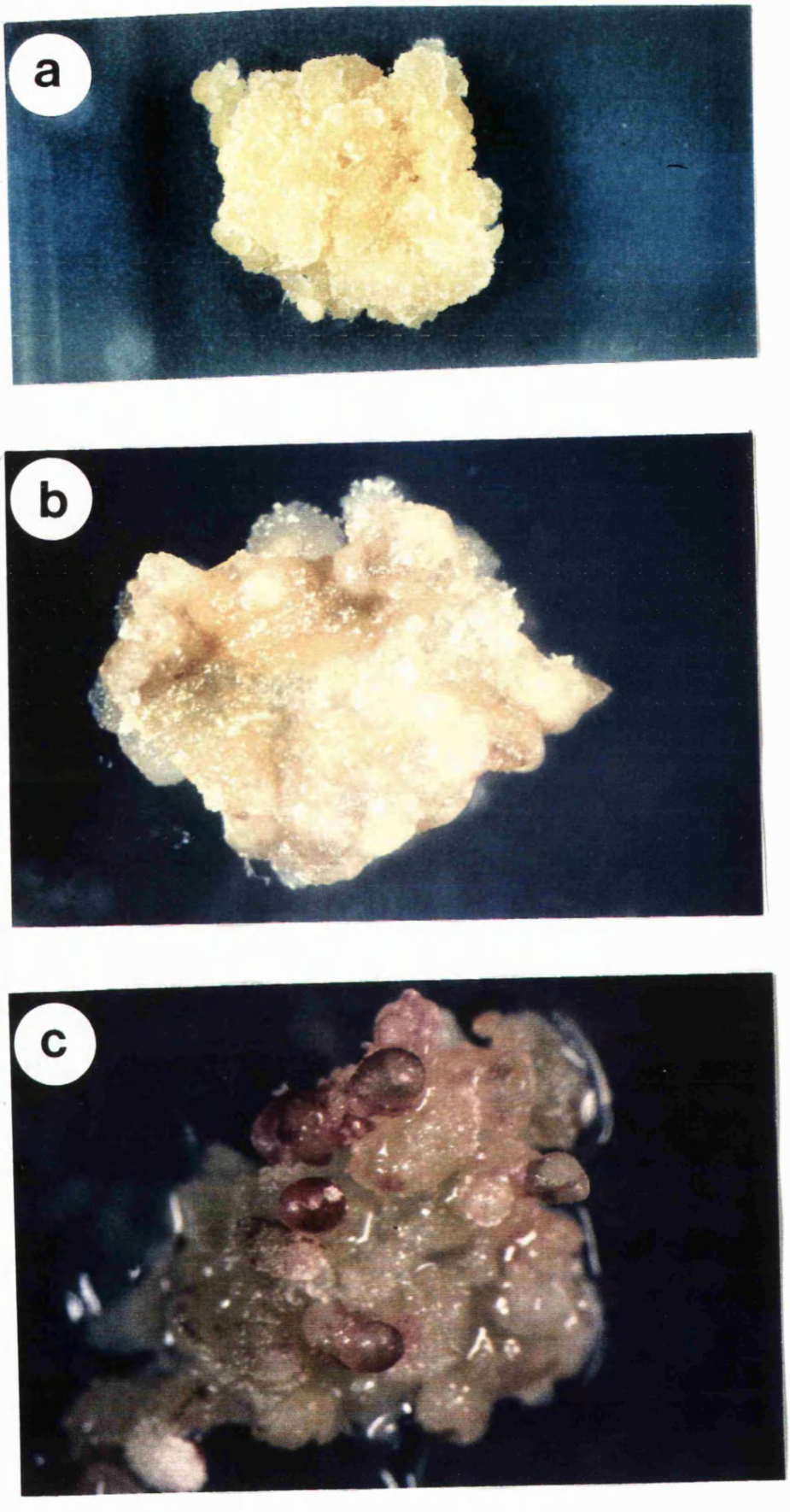


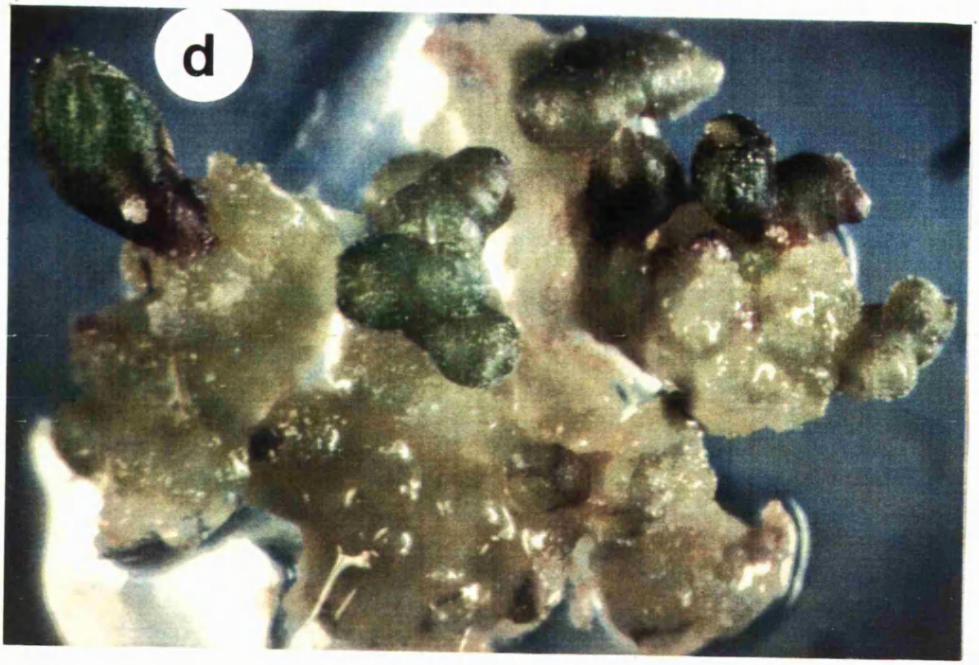
Fig. 62.

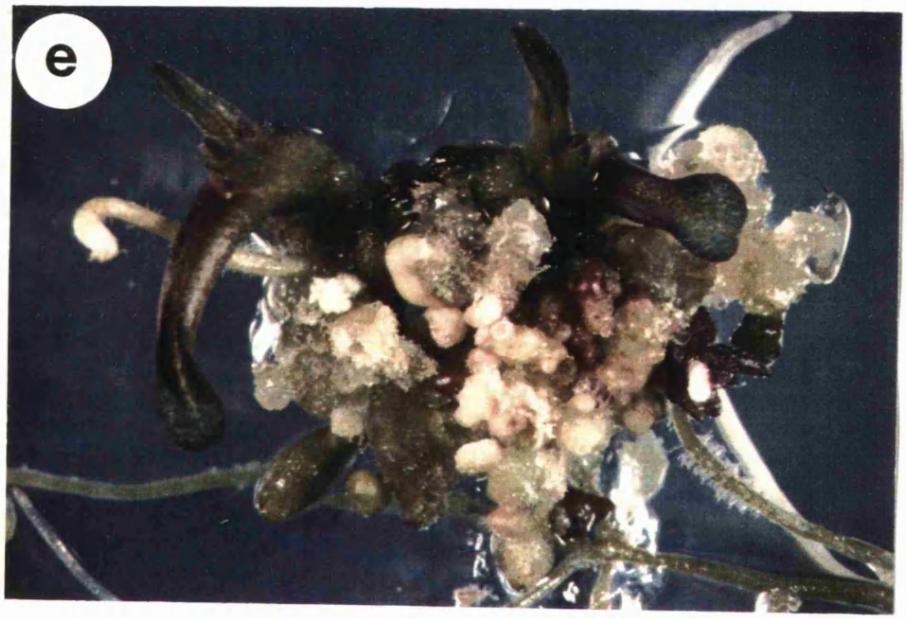
d. Shoot-buds development 4 weeks after transfer of callus with nodules to MS medium (hormone-free) supplemented with 6% (w/v) sucrose (Mag x 3).

e. Further development of shoot-buds 6 weeks after transfer to the MS-medium (hormone-free) with 6% (w/v) sucrose (Mag x 2).

f. plantlets development 8 weeks after transfer of shoot-buds to half strength MS-medium (hormone-free) with 3% (w/v) sucrose (Mag x 2).

Fig-62





The plant regeneration system from leaf callus in genotype HIB 687 can be summarised as follows:

Stage I: Callus induction from leaf explant on MS medium with 2,4-D 1.0 mg/l, 30 g/l, sucrose, three weeks incubation at 27 °C in the dark (Fig 62a).

Stage II: Callus transferred to MS medium with 30 g/l sucrose and RB 10 mg/l + DHT 100 mg/l or DHT 100 mg/l; four weeks incubation at 25 °C, 16 hr photoperiod (30 $\mu Mm^{-2}s^{-1}$APR), some nodules were formed (Fig 62b).

Stage III: Callus transferred to MS medium with 30 g/l sucrose and Zn 0.25 mg/l + 1AA 0.5 mg/l;, four weeks incubation under same conditions as for stage II. Numerous nodules and dormant shoot-buds (Fig 62c).

Stage IV: callus (with nodules from stage II) transferred to MS with high sucrose (60 g/l) for six weeks, incubation under same conditions as for stage II. Vigorous shoot-buds formed (Figs 62d & 62e).

Stage V: shoot-buds transferred to half strength MS medium with 50 g/l sucrose or MS with 30 g/l sucrose; eight weeks incubation under same conditions as for stage II. Shoot-buds developed roots and became plantlets (Fig 62f).

The regeneration system produced plants that could be acclimatized, potted in soil and transferred to greenhouse in approximately seven months.

6.3.2.3.4 Results from Tests for Virus Elimination:

60 plants regenerated through the above system that were subjected to a virus indexing procedure (section 6.2.2.4), but only eight plants were found to be on the

borderline for negative readings, and even these would require a second stage testing to confirm their virus infection state. It was therefore concluded that, no virus elimination had taken place during regeneration.

6.3.3 Callus Induction from Isolated Leaf cells

6.3.3.1 Introduction

The aim of the experiment was to capitalise upon the fact that sweet potato leaf cells can be relatively easily separated mechanically and, thus, produce a relatively simple procedure for cloning from single cells. Such a regeneration technique would be useful in combination with direct DNA delivery by particle gun transformation, and could lead to developing virus resistance in sweet potatoes through genetic engineering. This would avoid some of the problems that can arise from the need to use enzymes and osmotica for the isolation of protoplasts, such as somaclonal variations.

Although a few successful attempts have been made by other workers to isolate leaf cells of sweet potato, either mechanically or enzymatically, the techniques used were generally long and complicated and they often produced small, weak callus colonies, with limited growth.

Bhatt *et al.* (1974) reported mechanical isolation of leaf mesophyll cells from *Ipomoea guamoclif* L. by hand maceration, with 1% of cells developing into callus colonies. Bidney *et al.* (1980) used enzymatic isolation of mesophyll cells and protoplasts by a three media design cell culture system to achieve callus development. Schwenk (1981) reported the mechanical isolation of sweet potato mesophyll cells by use of a vortex impinger and a culture procedure which produced limited callus growth on a medium with NAA.

Attempts on enzymatic isolation and plant regeneration from mesophyll

protoplasts, as compared to leaf cells have been relatively more successful, although they may or may not be relevant because of their different isolation and culture requirements. Sihachakr *et al.* (1987) using enzymatic isolation of mesophyll protoplasts on a culture medium with NAA, 2,4-D and Zn, achieved some limited (5%) but significant success because it was the first report on plant regeneration from this type of callus system. Murata *et al.* (1987) also had callus formation and plant regeneration from petiole protoplasts by using a combination of Kn, 2,4-D, ABA & BAP. Kokubu *et al.* (1988) reported successful isolation and culture of petiole protoplasts of sweet potato and related species for protoplast fusion, and achieved cell division using a modified cell-layer medium with 2,4-D and Kn. Murata *et al.* (1989) reported successful protoplast isolation callus formation and shoot regeneration from petiole protoplasts by means of a medium containing Kn and indicated that shoot formation was enhanced by the nitrogen compounds glutamic acid, asparagine and proline.

6.3.3.2 Isolation of Cells from Leaves

Three sweet potato genotypes 157 , cvs. Papota and HIB 687 maintained in the greenhouse as described in section 2.2, were used for the study.

The second fully expanded leaves from the growing tip of greenhouse-grown plants were collected and weighed after mid-ribs had been removed. Five grams of leaf tissue were placed in a plastic beaker surface sterilized as described in section 2.4. The leaves were then placed in a 9 cm sterile Petri dish (Sterilin) and cut with sterile surgical blades into 2 cm squares which were transferred to a glass beaker and mixed with isolation medium (IM) (half strength liquid MS basal medium with 2% sucrose, without supplements, Table 36). 25 ml isolation medium containing 1.7 g leaf sections were added to a glass homogeniser (Jencon Ltd.) and macerated

mechanically at room temperature in a laminar flow cabinet, until no leaf pieces could be seen. The homogenate was collected in a glass beaker and filtered first through a one mm muslin cloth, then through a 100 μm nylon mesh 20 ml of the filtered homogenate was poured into 30 ml capacity centrifuge tubes (Nalgene Ltd, USA) which were centrifuged (Du-Pont Sorvall RC-SB) at 10 °C, and 1000 rpm for 10 minutes.

The supernatant was gently poured off and the cell pellet re-suspended in 20 ml IM, and the process repeated twice. The final pellet was collected in a 20 ml beaker and re-suspended so that cells from 1 gm of leaf tissue were collected in 2 ml IM. The leaf cell density was determined using a haemocytometer.

6.3.3.3 Cell Plating

Six cell plating densities were employed with cell plating medium (Table 36): $1x10^4$; $3x10^4$; $5x10^4$; $1x10^5$; $1.7x10^5$ and $3.4x10^5$ cell/ml. A sample of the cells was taken using a pipette (Blowout) and gently added to the cooled (30 °C) plating medium (Table 36) in a glass beaker, while gently stirring the mixture with a magnetic flea and stirrer. The mixture was dispensed so that a 10 ml aliquot was added to a 9 cm Petri dish (Sterilin single vent) which was sealed with parafilm (Parafilm M USA) and incubated in a growth chamber at 27 °C in the dark for 7 days, after which they were removed from the growth chamber, and observed under the microscope for cell divisions. They were then uncovered and 2 ml per Petri dish of liquid cell plating medium was added; after re-sealing they were incubated in a growth room at 25 °C and 16 hr photoperiod ($30\mu Mm^{-2}s^{-1}$PAR.

After four weeks 5 mm diameter colonies were transferred to the different media being tested for regeneration, contained in 9 cm Petri dishes; 50 calluses were cultured in each Petri dish, and ten Petri dish replications were made for each test

medium. The cultures were scaled with parafilm and incubated for four weeks in the growth room in the conditions described in section 2.4. MS basal medium supplemented with 30 g/l sucrose, 50 mg/l casein hydrolysate, 0.5 mg/l folic acid, 0.05 mg/l biotin, 2.0 mg/l calcium pantothenate, different combinations of Zn and 1AA and 7.0 g/l agarose (Sigma Type I) were used for the regeneration tests (Table 37). The hormones were tested alone in combinations at the following concentrations: 0, 0.25, 0.5, 1.0, 2.0 mg/l, producing 25 different test media (MI to M 25 in Table 37).

Table 36. COMPOSITIONS OF CELL ISOLATING AND PLATING MEDIA

Basal Medium: MS (Table 6) half strength, pH 5:6				
	Cell Isolating		Cell Plating	
	mg/l	g/l	mg/l	g/l
NAA	-	-	0.5	-
2,4-D	-	-	0.2	-
Zn	-	-	0.5	-
Folic acid	-	-	0.5	-
Biotin	-	-	0.05	-
Calcium pantothenate	-	-	2.0	-
Casein hydrolysate	-	-	50.0	-
Sucrose	-	20.0	-	20.0
Agarose (Sigma Type I)	-	-	-	5.0

Biotin, folic acid and calcium pantothenate were filter sterilized.

6.3.3.4 Results

Observation of the isolated cells under the microscope indicated that the use of the glass homogeniser was a very effective method of producing high densities of intact mesophyll cells and the various cell types could be readily distinguished they

intact mesophyll cells and the various cell types could be readily distinguished they had retained their cell walls, and original shapes. The had maceration effectively broken the cell-to-cell attachment and the mixture was essentially of single cell units (Fig 63a).

From the three genotypes cultured, only 157 responded with cell divisions leading to callus development and by the 7th day, it was observed to have vigorous cell division (Fig 63b). Cell division was observed at all of the six plating densities, but callus colonies developed only with the four higher plating densities, from $5x10^4$ cells /ml. At cell densities $1.7x10^5$ and $3.4x10^5$ cells/ml, the closely packed mass of proliferating cells colonies made it difficult to isolate individual colonies for transfer to secondary media and planting densities of $5x10^4$ and $1x10^5$ cells/ml were considered to be the most suitable for further work. At the end of the fourth week, 5 mm diameter cell colonies had developed and they could be used for secondary transfers for the regeneration experiments.

Each leaf cell that responded with cell division developed to a green single cell callus colony (Fig 63c) and where several single cell colonies could not be separated due to high cell densities, they grew together with loose association (Fig 63d). It was possible to pick individual 5 mm diameter colonies easily for transfer to the regeneration media.

Table 37 describes in detail the morphogenetic responses induced on the test regeneration media. The results indicated that Zn was essential for the development of chlorophyll in the callus, but alone it produced only friable callus. In appearance, the most promising callus for regeneration was dark green, slow growing and compact (Figs 63e & f) was obtained with treatment M12 (Zn 0.25 mg/l + 1AA 0.5 mg/l);

Table 37. EFFECTS OF ZEATIN AND 1AA ON GROWTH AND DEVELOPMENT OF SWEET POTATO MESOPHYLL CELLS-DERIVED CALLUS

Zn (mg/l) 1AA (mg/l)	0	0.25	0.5	1.0	2.0
0	(MT) 0 brown compact	(M2) ++ Green friable	(M3) ++ Green friable	(M4) +++ Green friable	(M5) ++ Green friable
0.25	(M6) 0 brown compact	(M7) ++ green compact	(M8) ++ light-green friable	(M9) +++ Green friable	(M10) +++ Green friable
0.5	(M11) ++ creamy friable	(M12) ++ dark-Green compact	(M13) ++ Green friable	(M14) +++ Green friable	(M15) +++ Green friable
1.0	(M6) +++ yellow friable	(M17) +++ light-Green friable	(M18) +++ Green friable	(M19) +++ Green friable	(M20) + Green friable
2.0	(M21) +++++ white friable	(M22) ++++ creamy friable	(M23) +++ yellow friable	(M24) +++ light-Green friable	(M25) ++++ orange friable

+= 5 mm diameter callus growth, ++= 10 mm, +++= 15 mm, ++++= 20 mm.

0= No callus growth

Inoculum: 5 mm callus from leaf cells of genotype 157 (section 6.3.2.1).

Basal medium - MS + 30 g/l sucrose

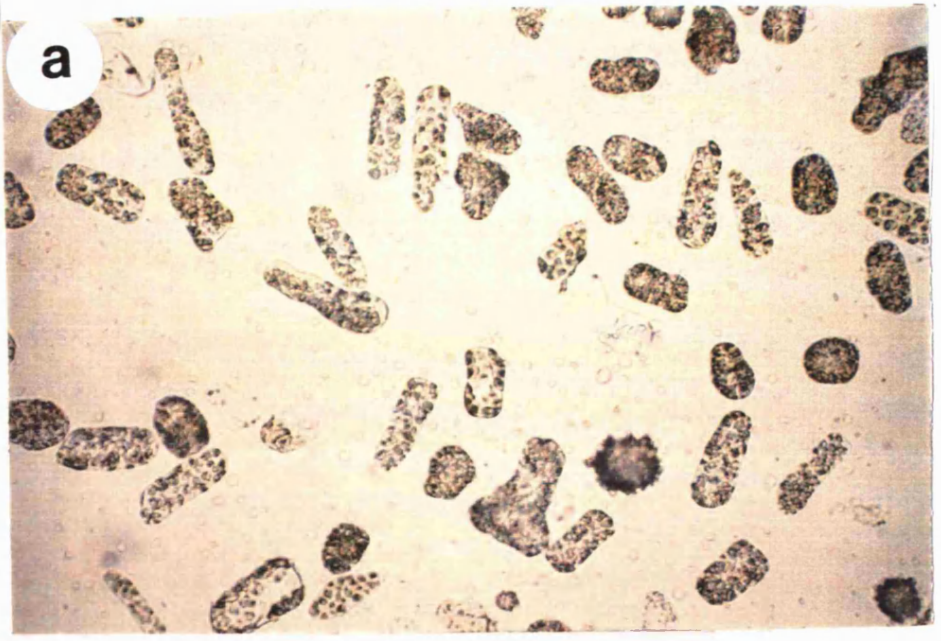
Replicates: 500 calluses

Incubation conditions: 4 weeks at 25 °C, 16 hr photoperiod (30 $\mu Mm^{-2}s^{-1}$PAR)

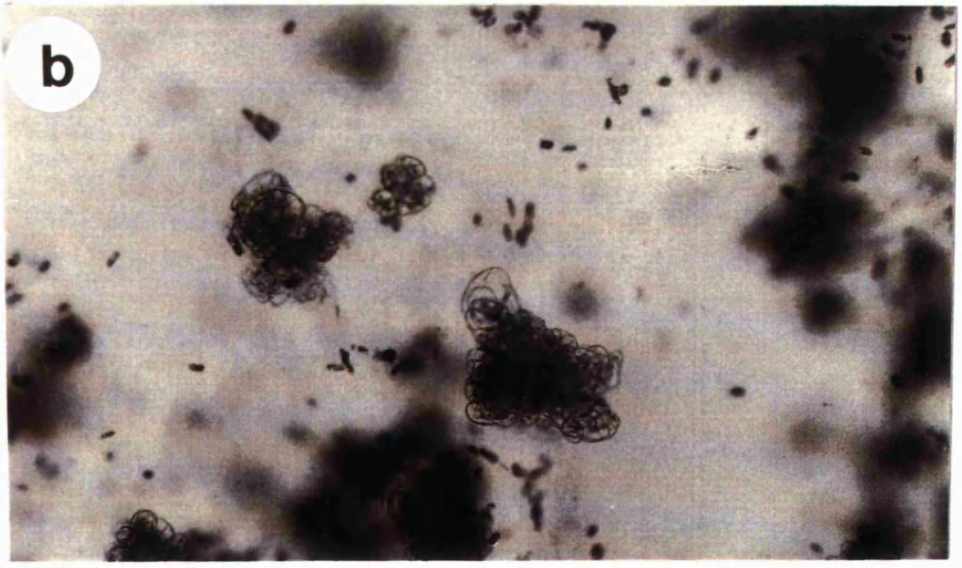
Fig. 63. Callus formation from isolated leaf cells.

63I. Cell colony formation from isolated leaf cells, cultured on plating medium (Table 36) and incubated first for one week in a growth cabinet (27 °C in the dark) then for three weeks in a growth room (25 °C, 16 hr photoperiod 30 $\mu Mm^{-2}s^{-1}$PAR).

g.63



a. inoculum (cells) at day zero (Mag x 372).



b. Small colonies 10 days after plating (Mag x 380).

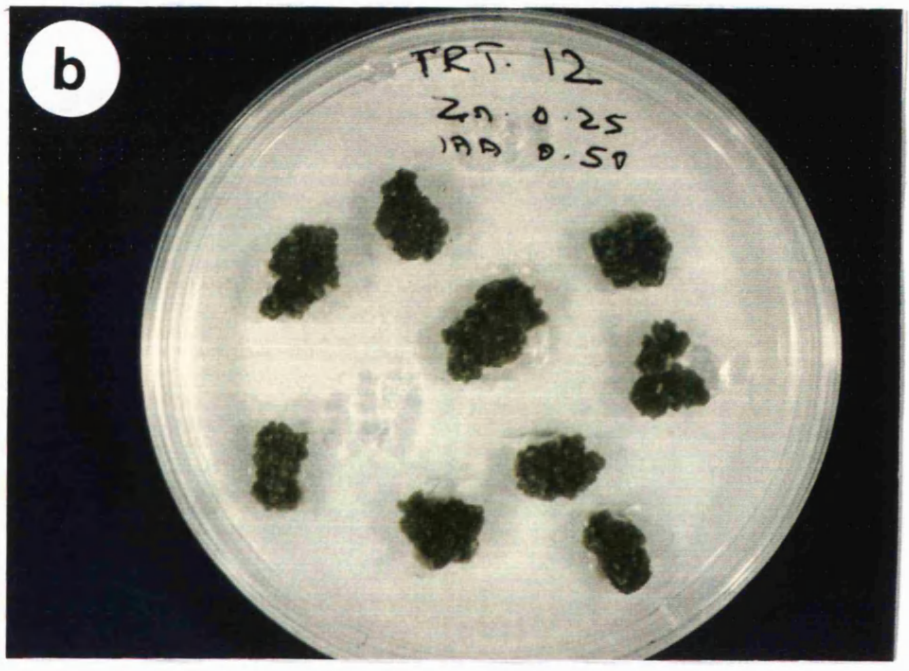
63II. Colonies formed from isolated leaf cells, after calluses were transferred from plating medium to regeneration medium (M12, Table 37), and incubated in growth room.

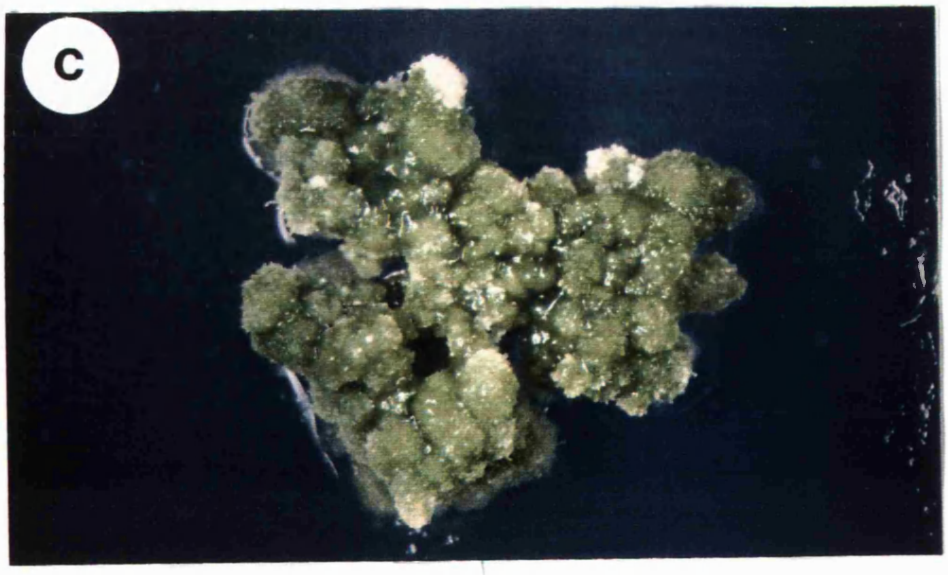
a. 5 mm colony at day zero (Mag x 15).

b. Colonies development in a plate after 4 weeks of incubation (Mag x 1).

c. Details of one callus colony from 6 (Mag x 3).

Fig-63-II





Summary of procedures leading to callus development from leaf cells:

Stage I - mechanical maceration of leaf sections in a glass homogenizer with isolating medium (Table 36).

Stage II - Isolating of leaf cells by filters and centrifugation.

Stage III - Cell plating (and testing the suitable plating densities) by mixing with plating medium (Table 36) for cell division and callus induction.

Stage IV - Transfer of single cell callus colonies (5 mm diameter) or several colonies together, on test media for plant regeneration (Table 37).

6.4 DISCUSSION

6.4.1 Effects of Chemotherapeutants

This section includes results of studies undertaken to develop methodologies for plant regeneration in sweet potato, from roots leaves and leaf cells, via derived callus cultures, for use with chemotherapy for possible virus elimination. Successful plant regeneration was achieved from roots although this work did not include the use of chemotherapy; with leaf-derived callus cultures with the use of hormones, chemotherapeutants and high sucrose treatments led to plant regeneration, but without achieving virus elimination. Preliminary investigations with isolated leaf cells showed that callus colonies with a possible potential for plant-regeneration could be produced. Strong genotypic influence was observed as success was only achieved with one out of the three genotypes attempted in all cases. The role of the chemotherapeutant in assisting shoot regeneration from leaf-derived callus cultures was an unexpected observation, especially as most reports, including those in sections 4.3 and 4.1 associate *in vitro* chemotherapy, with considerable growth inhibitions and even phytotoxicity. Lozoya *et al.*, (1984), working on the eradication of TMV from tobacco callus using RB (0.22 mg/l) & VID (0.027 mg/l) reported that success was only achieved with 50% inhibition of callus organogenesis when compared to control cultures. Cassells *et al.* (1980) reported growth inhibition by RB (10 mg/l) which he used in potato callus cultures to eradicate cucumber mosaic virus (CMV). Simpkins *et al.*, (1981) reported growth inhibition and even toxicity, when they screened five different chemotherapeutants which included RB in order to eradicate CMV and alfalfa mosaic virus from tobacco callus. Dawson, (1984), in a search for plant antiviral agents, screened 27 chemicals, found 14 to have plant antiviral effects, and that all 14 chemicals exerted some amount of growth inhibition on the host plant, which was different for each compound. 2-Thiouracil, Vidarabine and Ribavirin were

included in the 14 chemicals which were tested on TMV infected tobacco callus. Reports (Schuster *et al.*, 1984; Bittner *et al.*, 1986) on the antiviral effects of DHT on callus cultures fail to mention whether it inhibited growth, but work on shoot-tip cultures and *in vivo* sprays indicate that DHT does cause some growth inhibition, although to a lesser extent when compared to Ribavirin, Vidarabine and thiouracil. Schuster, (1982) reported the combined use of DHT and RB succeeded in suppressing the symptoms of PVX, TMV, CMV and BMV in tobacco when sprayed on to field-growing plants, but the treatment also caused growth retardation of the plants.

In the present studies, 2-thio uracil alone (R14) (see also section 4.3) or in combination with Vidarabine (R15 section 6.3.2.3.1) inhibited the growth of calluses resulting in death for all genotypes. The same combination of VID (0.027 mg/l) with RB 0.22 mg/l (R18 section 6.3.2.3.1), that was used by Lozoya *et al.*, (1984) on tobacco, inhibited callus growth just like 2-thio uracil. VID alone (R16 and R17 section 6.3.2.3.1) inhibited the growth of callus but one genotype H1B 687 was not affected and developed roots when transferred to MS basal medium, indicating there was genotypic influence.

In the present studies, RB and DHT, either alone or in combination, were not observed to inhibit callus growth for any genotype (section 6.3.2.3.1, Table 35). RB combination with DHT actually exerted a beneficial effect which differed in different genotypes, but induced roots formation (R10 and R13) in Papota and genotype HIB 687. Further, plants were only regenerated from callus that had been cultured first on the media containing RB 10 + DHT 100 mg/l or DHT 100 mg/l (Fig 62b) alone before transfer to medium with Zn 0.25 mg/l + 1AA 0.5 mg/l for four weeks, and finally to MS basal without hormones, but with 60 mg/l sucrose, as described in Figs 61a-f, section 6.3.2.3.3.

However, Stone (1982) reported Vidarabine (1 mg/l) stimulated growth in

Ullucus tuberosum meristem-tip cultures and also increased the rate of viral elimination. This report is supported by the observation here of growth stimulation while using vidarabine with meristem-tip culture (Table 25, Figs 53, 54a,b,c, section 4.3), but the virus elimination was not significantly greater than with the control. Perhaps the closest report to our observation was that made by Simpkins *et al.*, (1981), who reported that, RB (10 mg/l) increased the fresh weight of tobacco callus cultures after 24 days of incubation. They also reported virus suppression as a result of Ribavirin treatment. In the present studies, virus elimination was not observed, but this is possibly because organogenesis occurred on transfer to medium without chemotherapeutants, whereas Simpkins *et al.*, (1981) and Cassells *et al.*, (1982), both attempting to eliminate viruses from tobacco and potato callus cultures, respectively, by use of RB, observed that, virus elimination was only effective when organogenesis occurred in the presence of the chemotherapeutant. This would mean that the effective hormones and chemotherapeutants would have to work combined in one medium during the organogenesis stage, however a similar attempt with sweet potato was not successful section 6.3.2.3.2. The role of chemotherapeutants in assisting regeneration in these treatments, was possibly the result of a suppression of the carry-over effects of 2, 4-D, allowing Zn and 1AA to be more effective in the next stage.

6.4.2 Plant Regeneration from Root Callus

Ipomoea batatas is one of the plant species where shoots develop readily from root tubers *in vivo*, and it was important to investigate this potential *in vitro*, for possible use with virus elimination systems or for possible use in developing virus resistant cultivars through genetic transformation system utilizing for example a *Agrobacterium rhizogenes*.

In this work, shoot regeneration was achieved with kinetin (0.2 mg/l and 0.4

genotypic influence. However, shoots were regenerated directly from the primary callus, induced from the original root explants (Fig 60, section 6.3.1), in a system that is likely to produce genetically stable clones. Comparable reports with this type of regeneration with sweet potato could not be found in the literature. However there were a few reports, one from Tsai *et al.,* (1973) describing organ differentiation from tuber-derived callus using BA, 1AA, and ABA, and indicating that success was genotypic dependent; and the other two from Hwang *et al.,* (1980) and (1981) describing successful plant regeneration from callus derived from internal regions of tuberous roots, using BA and NAA, and again indicating the genotypic effects.

6.4.3.1 Plant Regeneration Following the Use of NAA, BAP and GA_3

Regeneration here was achieved by use of the hormones NAA, BAP, and GA_3, followed by a transfer to hormones-free MS basal medium with high concentration of sucrose (60 mg/l). This system of plant regeneration was associated with strong root formation, and shoot buds only occurred at the base of vertical adventitious roots (Figs 61a-d, section 6.3.2.2). The red nodules, which developed to form dormant shoot-buds developed further to form shoots and plantlets when isolated and cultured independently on hormone-free, MS basal medium with 60 mg/l sucrose. Carswell *et al.,* (1984) had a similar report on successful plant regeneration, from leaf-derived callus with, NAA and BAP but they did not use GA_3, and observed that regeneration was strongly associated with root formation and influenced by the genotype. GA_3 seem to have influenced the growth of shoot-buds once they were formed, but had no effect on regeneration. More recently Liu *et al.,* (1990) also reported a similar plant regeneration system, which occurred in association with root development from callus derived from leaf, petiole and stems of *Ipomoea triloba* L., a related species of sweet

potato. In their studies, callus was induced with 2,4-D and Kn, while regeneration was achieved with 1AA, and BAP, followed by hormone-free MS basal. It was further observed that callus from different explant sources had different levels of hormone requirement; and in the case of the leaf explant 1AA was not required for regeneration to occur. Such reports are widening the scope of explants and hormones that can be used for successful plant regeneration in sweet potato.

6.4.3.2 Plant Regeneration Following the Use of Zn and 1AA, and Chemotherapeutants (DHT and RB)

The successful direct shoot regeneration from leaf-derived callus, (without an association with root formation or somatic embryogenesis) reported from these studies, using Zn, 1AA, and enhanced by chemotherapeutants DHT and RB, was a significant contribution, and no comparable reports were found in the reported literature (Table 35, Figs 62a-f, section 6.3.2.3.3). The use of Zn as a cytokinin to effect plant regeneration from sweet potato callus was only reported by Sihachakr *et al.* (1987); most reported regeneration systems have used cytokinins Kn or BAP, combined with the auxins 1AA or 2, 4-D. Callus induction and embryogenesis were especially associated with the use of 2,4-D. Liu *et al.,* (1984) also had somatic embryogenesis from leaf callus by use of 2,4-D and a high sucrose concentration (60 mg/l) and noted that whole plants were regenerated only after a final transfer to hormone-free MS basal medium. Jarret *et al.,* (1984) succeeded in obtaining somatic embryos from axillary bud tips by use of 2, 4-D followed by a final transfer to hormone-free medium. Tsai *et al.,* (1979) reported embryoid formation from anther callus by the use of 2,4-D, Kn and 1AA, and noted the strong genotypic influences. Sehgah (1975) had regeneration of plants from leaf callus by use of adenine, and in 1978 reported plant regeneration from anther cultures. DeWald *et al.,* (1988) also had

1978 reported plant regeneration from anther cultures. DeWald *et al.,* (1988) also had successful embryogenesis from sweet potato callus derived from shoot apices, by use of 2,4-D. Xue (1987) reported successful plant regeneration from cotyledon-derived callus by use of 2,4-D and this was followed in 1988 by the successful plant regeneration from ovule-derived callus by use of 2,4-D, 1AA and cytokinin. Murata *et al.,* (1989) used of kinetin and amino acids for promotion of regeneration from protoplast-derived callus. Liu *et al.,* (1990) used 2,4-D, 1AA, and BAP to regenerated plants from petiole, leaf and stem explant-derived callus. Chee *et al.,* (1988) reported the use of 2,4-D and BAP in selective proliferation of embryogenic and non-embryogenic, callus and in 1990 also had successful somatic embryogenesis by use of NAA and BAP.

In the present investigation, the pathway by which organogenesis occurred and shoot-buds regenerated appeared to be new. Organogenesis started by the development of small red-pigmented nodules about one week after transfer to medium with DHT and RB. These nodules were not observed in cultures incubated either in the dark or on medium with 2, 4-D. If the callus produced in the presence of DHT and RB was not transferred to another medium, or was transferred to MS with 60 mg/l sucrose the nodules developed into adventitious vertical roots (Table 35). However, if the callus was transferred from DHT and RB medium to Zn + 1AA medium the nodules developed an intense red-purple pigmentation confined mainly to the nodules (Fig 62c) in about 80% of callus. Observations indicated that the shoot-buds developed from these nodules which were formed independently of roots; it was not unusual to find an entire callus regenerating shoot-buds (Figs 62d,c). Occasionally a few thin horizontal roots emerged from the side of the callus and developed away from the developing shoot-buds (Fig 62f). In these studies, nodules and dormant shoot-buds induced in the Zn, 1AA medium did not develop to

shoot-buds if they were not transferred to the hormone-free MS medium. This observation has been reported in almost all the regeneration systems with sweet potato. Perhaps this requirement is explained through the work of Chee *et al.*, (1989). In a study of the mechanism by which development from embryogenic callus to embryo development was inhibited, Chee found that, the added exogenous auxins disrupted the efflux of endogenous 1AA from embryogenic loci. This seemed to indicate that, in these cases the exogenous hormone are only needed to stimulate certain developmental processes, after which their presence may become inhibitory.

6.4.4 Mesophyll Cell Isolation and Callus Development:

The successful but simple techniques developed for sweet potato mesophyll cell isolation, and the good callus development that was achieved, was a significant contribution to the cell culture procedures available for sweet potato (Figs 63a-f, section 6.3.3.4). Although a few successful attempts had been made to isolate sweet potato mesophyll cells either mechanically or enzymatically the techniques reported were often long, somehow complicated, with complex media designs, and often produced some small and weak callus colonies with limited growth (Bhatt *et al.*, 1974; Bidney *et al.*, 1980 and Schwenk, 1981).

In these studies, cell isolation was achieved by use of simple glass homogenizer, with half-strength MS basal medium containing 20 g/l sucrose as the isolation medium, and one gram of sweet potato leaf tissue yielded about $1.7x10^7$ cells in intact form (Fig. 63 a). The optimum cell-plating density for good callus colony growth of $5x10^4$ cell/ml, produced cell colonies that formed green compact tissues, particularly in the presence of Zn and 1AA (Table 37) from their appearance, the culture might have had the potential for regeneration but time did not permit further investigation. It was worth noting that it was a similar Zn (0.25 mg/l) 1AA (0.5

6.3.2.3.3), but did not stimulate any effects on the genotype (HIB 687) used here, but rather influenced a different genotype (157) during those studies.

Probably the most comparable work was that reported by Schwenk, (1981) (section 6.3.3.1) who reported successful isolation of sweet potato mesophyll cells by use of a vortex impinger, an optimum cell planting density of $5x10^5$ cells/ml, but only achieved limited callus growth on medium with NAA. Bhatt *et al.*, (1974) reported mechanical isolation of mesophyll cells from *Ipomoea quamoclif* L. by hand maceration, with 1% of cells developing into callus colonies. Bidney *et al.*, (1980) used enzymatic isolation of protoplast and mesophyll cells and a three media culture system to achieve callus development. Otani *et al.*, (1987) used enzymatic isolation of mesophyll protoplasts and medium containing 2,4-D and ABA, but only achieved limited callus development. Sihachakr *et al.*, (1987) used enzymatic isolation of mesophyll protoplasts and a culture medium with NAA, 2,4-D and Zn, to achieve some limited (5%) but significant plant regeneration from callus. In the present attempts at regeneration, the Sihachakr *et al.*, (1987) medium was used, but without any success, probably due to genotypic differences. Murata *et al.*, (1987) reported successful callus formation and plant regeneration from petiole protoplasts of sweet potato, by use of modified MS medium with Kn to stimulate callus formation; modified MS plus 2,4-D, ABA and Kn to induce shoot formation; and Kn with BAP to induce root formation. More recently, Murata *et al.*, (1989) reported on the improvement of shoot regeneration from protoplast-derived callus cultures of sweet potato, by use of Kn, and further supplementation of modified MS medium, with the amino acids and amides glutamic acid, asparagine and proline.

CHAPTER 7

GENERAL CONCLUSIONS

7.1 Overall Aim of the Research

The overall aim of the research reported in this thesis was to obtain the essential information needed for the control of sweet potato viruses in Kenya. This, it was hoped, was to be achieved through three main objectives:

1. To develop more efficient *in vitro* techniques for obtaining virus-free plants from infected stocks.
2. To develop cell culture regeneration techniques to facilitate the elimination of viruses from infected stocks and their application to genetic engineering techniques for the production of virus resistant sweet potato cultivars.
3. To devise rational strategies for the control of virus spread in field grown elite virus-free sweet potato stocks.

7.1.1 Production of Virus-free Stocks

Two *in vitro* techniques (chemotherapy, chapter 4, and thermotherapy, chapter 5) were developed which doubled the efficiency of previously reported techniques for virus elimination. The *in vitro* chemotherapy described here is the first reported work with sweet potato; the use of both RB and DHT resulted in considerable improvement on the technique (MTC) previously often used (4.4.4). However, the "carry over" effects (see section 4.4.5) of both RB and DHT to stocks grown in the field the following season need further investigation. Both chemotherapy and thermotherapy need to be applied to provide virus-free sweet potato germplasm for various purposes in Kenya. Such virus-free sweet potato cultivars are needed, for example, for further epidemiological studies (see section 3.3.2). During these studies,

imported cultivars (section 3.2.1.2) were used because of the unavailability of virus-free Kenyan cultivars. Consequently, problems were encountered during the course of the field studies as some cultivars were not adopted to local conditions and, in some areas such as Kakamega, plants in the experimental plots were totally destroyed by weevils before their evaluation could be completed (see section 3.3.2.1). There is a need, therefore, to repeat the epidemiological experiments using virus-free Kenyan cultivars to see if they perform differently. The very poor performance of "improved" germplasm imported from international germplasm collections for crop improvement, clearly show the need for proper national evaluation trials before such material is distributed to farmers.

Virus-free cultivars are also needed for other studies such as the estimation of virus-induced yield-losses and an assessment of the economic importance of the viruses. Such studies have yet to be made in Kenya and are important in justifying the need and financial inputs for virus control programmes. Sweet potato breeders in KARI need virus-free germplasm, especially for accurate evaluation of performance trials.

Virus-free sweet potato stocks are also needed for germplasm conservation and thus prevention of losses caused by viral degeneration, especially of very susceptible but otherwise important cultivars. During the surveys (see section 3.3.1.1) it was observed that farmers grew very few cultivars (usually only one or two), and indicated that they previously grew more cultivars before their loss due to degeneration. This was further confirmed by the use of KARI collections (section 3.2.1.1) for epidemological experiments at Muguga. These collections contained many more cultivars than the farmers from whom they were originally collected; even at the Agricultural Stations, the officers in charge (personal communications) reported the loss of several important but susceptible cultivars due to weevils and

virus-induced degeneration. This further indicates the need for virus-free stocks for both field conservation of germplasm and also, perhaps for *in vitro* conservation as previously reported (Florkowski *et al.*, 1990; Huaman *et al.*, 1988; Jarret *et al.*, 1986 and Ng, 1986) and Cryopreservation (IBPGR, 1988 and Kadir *et al.*, 1989).

Interviews with the farmers on the origin of their sweet potato planting stocks (Table 9) revealed that such materials are usually obtained from neighbours, a procedure which also facilitates the perpetuation and distribution of viruses. It was interesting to note, however, that the commercial cultivar (Kalamu Nyerere, Table 9) commonly grown in the Kambondo area in South Nyanza had originated from Tanzania, and was introduced by farmers without any phytosanitary considerations. The occurrence of common virus complexes in Western Kenya and Central Uganda also indicated that there had been exchanges of germplasm between farmers in Western Kenya and Central Uganda; this emphasises the need for the international exchange of virus-free germplasm and virus control measures among neighbouring countries. The international exchange of sweet potato germplasm between distant countries in Africa is the madate of IITA (Frison, 1981; Ng, 1987), but would also require virus-free germplasm.

Replacement of the virus infected, degenerated, and low yielding stocks currently being grown by the Kenyan farmers by virus-free elite stocks would probably be the most important contribution from these virus elimination studies. Although the average national yield of sweet potatoes in Kenya is higher than that of neighbouring countries and other African countries (Table 5), it is still substantially below that in developed countries and China (Table 2). Considerable increases in production can probably be achieved by improvement of existing stocks through new technologies. Virus indexing of samples collected during the surveys (section 3.3.1.1) showed that viruses are widespread in sweet potatoes grown in Kenya, and often

occurred in complexes (Table 13). Using virus elimination techniques such as those described here (chapters 4 and 5), virus-free nuclear stocks suitable for particular regions of Kenya can now be produced and tested using any of the techniques described in sections 2.5.1, 2.5.2, 2.5.3, 2.5.4, and 2.5.5. Such certified germplasm could then be multiplied in isolated locations relatively free of virus vectors, or in high humidity screen houses, using nodal cuttings to produce sufficient quantities for replacement of relatively large areas to minimise re-infection (see section 3.3.2.1); this could be done region by region within a relatively short period. Such rapid field multiplication techniques have been developed by CIP (Dr. Carlo Carli, personal communication) and are currently being tested in dry areas of Kenya to provide sufficient planting stocks.

However, new technologies need to be introduced with caution and with a clear understanding of the sustainability of the proposed development. A feasibility study would probably be needed to determine how the new procedures will affect farmers, especially on the following issues: will they derive economic benefits, need extra inputs to achieve those benefits and will there be technical difficulties in integrating the new technologies (i.e. introduction of the new virus-free materials from tissue culture) and existing farming systems. The relative importance of the new technology to the small scale farmers in production and marketing also need to be assessed, especially to determine whether there will be a net yield increase, reduction in production costs, a premium price for the products and/or other marketing advantages. The possible contribution of new procedures in conservation of natural resources such as soil and water and effects on pesticide and fertilizer uses also need to be better understood.

7.1.2 Cell Culture Studies

Although a successful cell culture regeneration system was developed (chapter 6), the procedure did not also effect virus elimination (see section 6.3.2.3.4) for the possible reasons already discussed (section 6.4.1). However, regeneration procedures utilising root-callus (section 6.3.1) and leaf-callus (section 6.3.2.1 and 6.2.3.3) will probably be useful for genetically engineering virus resistant sweet potato cultivars, such as the use of coat-protein gene (CP-gene) resistance (Nelson *et al.*, 1988). Although the economics of replacement of infected germplasm with commercially produced virus-free sweet potato cultivars are unknown, the rapid rate of re-infection in the epidemiolgical studies (section 3.3.2.1) suggest that it is likely to be a short-term and an expensive operation; it is necessary, therefore, to consider long term alternative control strategies, such as the production of virus resistant cultivars, although this could prove difficult as sweet potato viruses in Kenya occurred in complexes of up to seven viruses (Table 13). However, CP-gene protection has a broad spectrum protective effect on viruses of the same group (Dr. Roger Beachy, personal communication).

However, it would be necessary to extend the regeneration procedures for use with important Kenyan cultivars since genotypic variations were observed; such regeneration studies could be undertaken in the tissue culture laboratories in KARI, although the advanced genetic transformation biotechnologies would require collaboration of international centres and other advanced laboratories with, perhaps, the financial assistance of international aid agencies such as EEC, ODA and USAID until Kenya will have her own facility for such work, such as the proposed centre for plant and animal biotechnology (CPAB) (Anon, 1991).

7.1.3 Control Strategies for Spread of Virus Diseases in Field

The surveys, in which the viruses were detected by immunoassay, confirmed that virus infection is prevalent in all major sweet potato production areas of Kenya. Although SPMMV and SPFMV have long been known to occur in the country, the occurrence of the other viruses and the virus-like agent have not hitherto been reported in East Africa. Individual isolates of SPMMV, SPFMV and SPC-LV did not apparently differ significantly from those described elsewhere; however, further studies on a range of isolates are necessary to determine if, and to what extent, strain variation might occur. Similarly, the other viruses now need to be isolated and, after characterisation, compared with isolates of the viruses occurring in other geographical areas; such comparisons with SPLV and SPRSV will be especially interesting as they have hitherto been reported to occur only in Asia or South Pacific, respectively. Of particular interest also would be the characterisation of the putative virus-like agent, especially as it interacts synergistically with SPMMV to induce severe diseases in sweet potato and *I. setosa*, and is apparently similar to the agent reported in Israel and West Africa to interact synergistically with SPFMV.

The control of viruses infecting sweet potatoes in Kenya in the long term will probably be based on the production of the resistant cultivars by conventional or novel breeding procedures. However, in the foreseeable future, virus-induced crop losses can probably best be minimised by the production and the distribution of elite virus-free stocks (see section 7.1.1).

The epidemiological studies clearly demonstrated that re-infection would be rapid if healthy plants were grown adjacent to, or interplanted with, infected stocks. Further epidemiological experiments are required, therefore, to develop rational and practical strategies for minimising rates of re-infection. Emphasis in such studies

should initially, perhaps, be on SPMMV which has a wide experimental host range, a vector (*Bemisia tabaci*) which is polyphagous and often occurs in large populations and, as described previously, interacts synergistically with the virus-like agent to induce severe debilitation of infected plants; thus, it is unknown if SPMMV occurs naturally in other plant species and, if so, which may be important foci of infection.

7.1.4 Conclusions

The major aim of this research program was to contribute to the identification and the solution of production and the marketing problems that cause sweet potatoes to be scarce and expensive in Kenya. During the surveys (section 3.3.1.1) farmers often emphasised the need for increased production and noted the constraints to production and the marketing (Table 9); the latter included land availability (especially in the Central Province), lack of suitable cultivars for new schemes at dry areas in Eastern Province, lack of pest and disease resistant cultivars (especially in Western/Nyanza and Coast Provinces where weevils cause serious damage), and the marketing difficulties in all provinces.

These studies indicate that replacement of virus-infected, degenerated stocks with virus-free elite materials from tissue culture could result in an increased production (section 7.1.2). Where land availability is a major constraint, as in Central Province, such an undertaking would probably have a major beneficial impact. The introduction of suitable cultivars for different ecological zones of Kenya might be facilitated by introductions from the international gene banks held by international institutes such as CIP, CATIE, AVRDC and IITA; there is, however, need for their proper field evaluation before being distributed to farmers and, thereafter, careful and continuous monitoring by virologists (see section 3.3.2.1). The KARI/CIBC

project in Kenya is largely concerned with the control of weevil damage (Mr. Kariuki, personal communication). These studies have highlighted the importance of virus diseases as major constraint to increased sweet potato production in Kenya and Uganda. Some suggestions have been made on possible minimisation of virus-induced crop losses, in the short-term through germplasm replacement (section 7.1.2) and long-term through the production and introduction of virus resistant cultivars (section 7.1.3).

Sweet potato marketing problems occur in the USA when sharp increases in supply coincide with storage and the transport problems, circumstances which lead to temporary market surpluses and low prices (Edmond, 1971); conversely, decreases in production result in increased prices. The same circumstances now cause similar marketing problems in Kenya. As an alternative strategy, increased production by the use of new cost-reducing technologies should encourage increased consumption and thus make sweet potato production profitable even at low prices. Home economists, school teachers, social workers and rural development workers can also help encourage higher levels of sweet potato consumption by disseminating information on its nutritional value (Table 3, Villareal, 1979a,b,c) and demonstrating the methods of preparing novel and attractive dishes. Non-sweet cultivars could also be introduced (Alman, 1987) and grown in the dry areas as an alternative to potato. Sweet potatoes could also probably be grown as commodity crop for processing to other products such as animal feeds (Lui, 1986) or to flour as a substitute for cereal flour in bread making (Horton *et al.*, 1989).

The specific problems that merit priority attention probably differ in different provinces of Kenya; this emphasises the need for client-oriented national research programmes that have both technical and social economic expertise. Such research areas would have been identified previously in the social economic studies undertaken

in 1989-90 by Dr. P. Ewell of CIP (Dr. P. Ewell, personal communication). However, the expansion of production and consumption of sweet potatoes in Kenya will not result from promotional campaigns, but by programmes that effectively result in increased production.

Appendix I

MAJOR CLIMATIC AND SOIL CONDITIONS AT THE FOUR EXPERIMENTAL LOCATIONS

	Location			
	Muguga	Embu	Kakamega	Katumani
Altitude (m above s.l.)	2170	1460	1590	1575
Ecological zone	Semi-humid	Humid	Humid	Semi-humid
Rainfall (mm) Oct.-Dec. March-May Total (yr)	260 500 9095	400 600 1100	distributed throughout the year 1900 mm	300 mm 400 mm 800 mm
Temperature (°C) Max. Min. Avg.	20.8 10.8 15.9	 20.7	24.3 13.8 20.1	24.9 13.7 19.5
Evapo-transpiration (Penman E.O)	1647	1810	2097	1790
Soil type *	Humic Nitosol	Dystric Nitosol	Eutric Nitosol	(Ferral) Chromic Luvisol
Crops (main)	Maize, beans, potatoes, sweet potatoes	Maize, beans, bananas, potatoes, sweet potatoes	Maize, bananas, cassava, sugarcane, sweet potatoes	Maize, beans, peagion peas maize, cassava, sweet potatoes

* FAO/UNESCO Designation

Appendix II
COMPANIES SUPPLING PRODUCTS USED IN THESE STUDIES

Products	Companies
Laboratory chemicals	Sigma Co. Ltd., UK.
Agar	Oxiod Co. Ltd.
MS basal medium	Flow Laboratory Co. Ltd.
Balances	Satorius. Mettler-Mettler Instruments-Zurich.
Plastic beakers	US Pat DES.
Glass beakers	Pyrex Co. Ltd.
pH-metre	Orion Research Digital.
Magnetic Stirrer	Gallenkamp Co. Ltd.
Waterbath	Jencons Scientific Ltd.
Laminar flow cabinet	Microflow pathfinder.
Scissors, blades, forceps, etc	Swann Morton Co. Ltd.
Filters	Flow Lab. Ltd.
EtOH, Tween 20, MaOCl	BDH Co. Ltd.
Nicotine	Synchemical.
Pynosect	30 Mitchel Cotts.
Malathion	Farm Protection.
Growth Chambers	Satchwell Duotronic.
Pipettes, Hips, Eppendorf	Eppendrof, Germany.
Distilled water	Fi-steem water still, Fisons.
Vortex mixers	Scientific Instruments, Bohemia, USA.

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